THE SECOND VIRIAL COEFFICIENTS OF SOME CYCLIC HYDROCARBONS

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Summary

This paper reports some measurements of the second virial coefficient B of *cyclo*propane in the temperature range 300 to 400 °K. It also gives some values of B for *cyclo*hexane and benzene, derived from critical analyses of the published vapour densities and P-V-T properties of these gases between 300 and 650 °K. In each case the results have been fitted to the relation

$$B=\alpha+\frac{\beta}{T}+\frac{\gamma}{T^2}+\frac{\delta}{T^3}.$$

The following conclusions can be drawn :

(i) The values of B for benzene and *cyclo*hexane are consistent with the effects of pressure on the enthalpies and heat capacities of the gases.

(ii) *cyclo*Hexane and benzene show large and almost equal deviations from the law of corresponding states for monatomic gases, but *cyclo*propane shows a much smaller deviation.

(iii) It is impossible to fit the second virial coefficients of benzene and *cyclo*hexane to the theoretical curve for a Lennard-Jones (12,6) gas. But they can be fitted to the curve for a (28,7) gas, and the associated force constants are physically reasonable. It appears that the interaction potential for *cyclo*propane molecules is intermediate between the (12,6) and (28,7) potentials.

I. INTRODUCTION

The isothermal behaviour of a gas is conveniently represented by the virial equation :‡

$$PV/RT=1+B/V+C/V^2+D/V^3+. . ., \qquad .... \quad (1)$$

where P and T denote the pressure and absolute temperature of the gas, V is its molar volume, and R is the molar gas constant. The quantities B, C, D, . . ., are referred to as the second, third, fourth, . . ., virial coefficients. They are complex functions of T, but are independent of P and V (see, for instance, Hirschfelder, Curtiss, and Bird 1954). At pressures of a few atmospheres or less the series (1) is rapidly convergent and the behaviour of the gas is then sufficiently defined by the second virial coefficient B.

An earlier paper from this laboratory (Hamann and Pearse 1952) described a comparative method of measuring second virial coefficients, and reported

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‡ This expansion of PV/RT as a power series in $1/V$ is usually attributed to Onnes (1902), but it had been suggested earlier by Thiesen (1885).

some values of B for *cyclo*propane between 300 and 360 °K. We have now extended the measurements for this gas to 400 °K. At the same time we have derived values of B for two other cyclic hydrocarbons, *cyclo*hexane and benzene, by analysing the published data on their vapour densities and P-V-T properties.

II. Sources of Data

(a) cyclo*Propane*

Hamann and Pearse (1952) measured the second virial coefficient of *cyclo*propane by a comparative method in the temperature range 30 to 90 °C. We have now repeated the measurements using an improved apparatus (Hamann, McManamey, and Pearse 1953), and extended the temperature range to 130 °C. The *cyclo*propane (medical grade) was supplied by Commonwealth Industrial Gases (N.S.W.) Pty. Ltd. It was fractionated in a low-temperature column and sublimed under vacuum to remove dissolved air. Its vapour pressure at 194·6 °K was 0·066 atm, compared with a value 0·069 atm found by interpolation in the tables of Stull (1947).

Table 1

The Second Virial Coefficient of Cyclopropane

Temperature (°K)	B (cm^3/mole)	
	Hamann and Pearse (1952)	Present Measurements
303·16	—363·9, —366·1	—375·0
313·16		—345·0
323·16	—321·6, —323·5	—325·0
333·16		—307·0
343·16	—281·6, —283·0, —281·9	—281·5
353·16		—266·0
363·16	—253·7	—251·4
373·16		—238·2
383·16		—223·2
393·16		—210·4
403·16		—200·5

The experimental method was the same as that described in the earlier papers, the pressure at various volumes being compared directly with the pressure of a reference gas, nitrogen, at the same volumes. The results are given in Table 1.

(b) *Benzene*

We have previously (David and Hamann 1958) analysed the volumetric data for this gas using the sources listed in Table 2. We discarded some other results on the grounds of internal or external inconsistencies (Allen, Everett, and Penney 1952 ; see Francis *et al.* 1952).

In our present calculations we have retained the data in Table 2, with the exception of the single value of Casado, [illegible]ie, and Whytlaw-Gray (1951).

In place of this we have taken the revised values published recently by Bottomley, Remmington, and Whytlaw-Gray (1958*a*, 1958*b*). We have also included the new results of Andon *et al.* (1957), obtained by an accurate and direct compressibility method, in the temperature range 340 to 393 °K. But we have omitted the data of Münze and von Weber (1957) because they are wholly inconsistent with the other results for benzene and, in fact, with the known behaviour of gases in general. Münze and von Weber concluded from their experiments that the second virial coefficient changes sign at the critical temperature T_c, whereas the correct inversion temperature should be nearly twice this (Guggenheim 1953; Hamann and Lambert 1954*a*, 1954*b*).

TABLE 2

SECOND VIRIAL COEFFICIENT OF BENZENE

Data used in original analysis (David and Hamann 1958)

Authors	Method	Temperature Range (°K)	Number of Values
Casado, Massie, and Whytlaw-Gray (1951)	Vapour density	295	1
Francis and McGlashan (1952)* ..	Compressibility	316–362	12
Hamann and McManamey (1952)* ..	Comparative compressibility	343–398	5
Lambert *et al.* (1949)†	Compressibility	319–359	7
Waelbroeck (1955)	Vapour density	331–348	7
Gornowski, Amick, and Hixson (1947) ..	Compressibility	533–628	13

* See Francis *et al.* (1952).

† The numerical values of B were taken from Roberts's (1947) thesis, Oxford University (see Lambert *et al.* 1949).

At the time of our original analysis we were unaware of the vapour density measurements of Steurer and Wolf (1938) and of Mund, Gerbaux, and Mornigny (1951). The first authors plotted their results in the form of a smoothed curve of $2B/V$ against T. This plot is probably most reliable in the middle of the temperature range (373·2 °K), and at that point we find $B=-873$ cm³/mole. Mund, Gerbaux, and Mornigny (1951) listed values of the apparent molecular weight of benzene at a pressure of about ¼ atm. By comparing their figures with the true molecular weight, we can derive the values: $B=-1018$ cm³/mole at 338·3 °K, and $B=-904$ cm³/mole at 373·2 °K. These three new results are included in the present analysis.

(*c*) cyclo*Hexane*

Waelbroeck (1955) and Lambert *et al.* (1949) have reported values of the second virial coefficient of *cyclo*hexane measured at low pressures in the temperature range 315 to 404 °K. Also Rotinjanz and Nagornow (1934) have published some *P*-*V*-*T* data obtained at rather higher temperatures (433 to 574 °K) and higher pressures (2 to 30 atm). We have derived values of B from their results by extrapolating the quantity $(PV^2/RT-V)_T$ to zero pressure.

III. Results

The results have not been tabulated; instead we have plotted them in Figures 1 and 2, and fitted them by the method of least squares to the arbitrary relation:

$$B=\alpha+\frac{\beta}{T}+\frac{\gamma}{T^2}+\frac{\delta}{T^3}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where the coefficients α, β, γ, δ are independent of the temperature. The values of the coefficients are listed in Table 3, and the corresponding curves have been drawn in Figures 1 and 2.

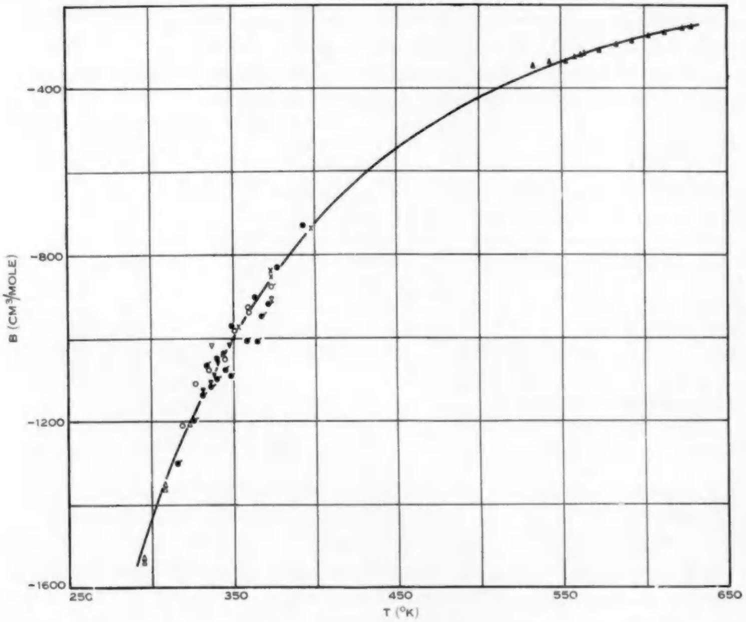

Fig. 1.—The second virial coefficient of benzene. The curve is defined by equation (**2**). Experimental points: ○′ Steurer and Wolf (1938); ▲ Gornowski, Amick, and Hixson (1947); ○ Lambert *et al.* (1949); ▽ Mund, Gerbaux, and Mornigny (1951); ●′ Francis and McGlashan (1952);* × Hamann and McManamey (1952);* ▼ Waelbroeck (1955); ● Andon *et al.* (1957); △ Bottomley, Remmington, and Whytlaw-Gray (1958*a*, 1958*b*).

It will be seen that the shape of the curve for benzene in Figure 1 is determined at one end by the low-temperature results of Bottomley, Remmington, and Whytlaw-Gray (1958*a*, 1958*b*), and at the other by the high-temperature results of Gornowski, Amick, and Hixson (1947). Its reliability therefore depends largely on the accuracy of these two sets of measurements. The first authors

* See Francis *et al.* (1952).

took considerable care to achieve accurate results and to overcome the effects of adsorption which can be misleading in measurements of B at low temperatures. Moreover, they obtained good agreement by two entirely different methods, so

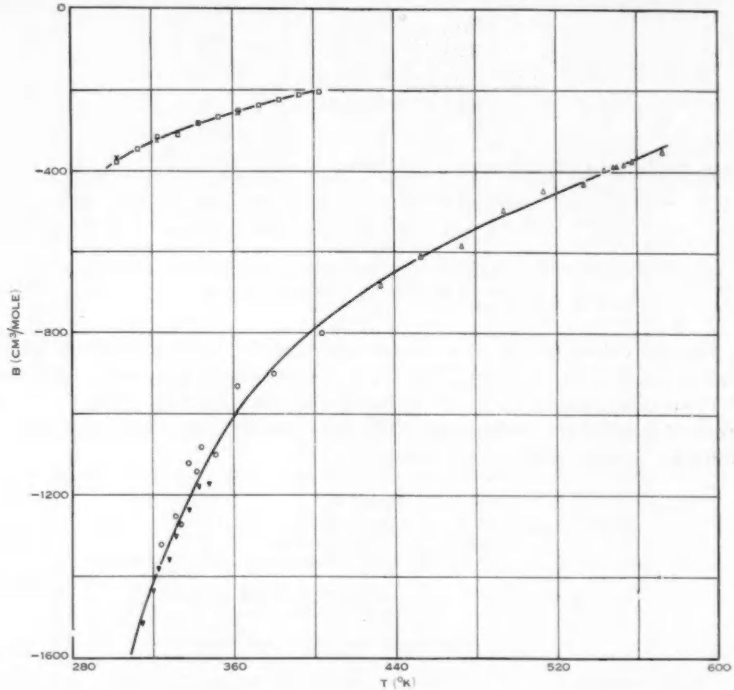

Fig. 2.—The second virial coefficients of *cyclo*propane (upper curve) and *cyclo*hexane (lower curve). The curves are defined by equation (**2**). Experimental points : × Hamann and Pearse (1952) ; □ present work ; △ Rotinjanz and Nagornow (1934) ; ○ Lambert *et al.* (1949) ; ▼ Waelbroeck (1955).

that their values can be considered reliable. It is more difficult to assess the work of Gornowski, Amick, and Hixson, but it is reassuring that their measured densities of saturated benzene vapour are close to those which Young (1910) had found many years earlier.

TABLE 3

THE COEFFICIENTS IN FORMULA (**2**)

B in cm³/mole, T in °K

Gas	α	β	γ	δ
*cyclo*Propane ..	$-7 \cdot 323 \times 10^{2}$	$+7 \cdot 925 \times 10^{5}$	$-3 \cdot 1289 \times 10^{8}$	$+3 \cdot 215 \times 10^{10}$
*cyclo*Hexane ..	$+3 \cdot 8595 \times 10^{3}$	$-5 \cdot 0986 \times 10^{6}$	$+2 \cdot 11617 \times 10^{9}$	$-3 \cdot 2822 \times 10^{11}$
Benzene	$-2 \cdot 288 \times 10^{2}$	$+3 \cdot 373 \times 10^{5}$	$-2 \cdot 2957 \times 10^{8}$	$+5 \cdot 95 \times 10^{9}$

A sensitive test of the empirical formula (**2**) is to see how well it describes the effects of pressure on the enthalpies and heat capacities of the gases at constant temperature. At low pressures these changes are given by the derivatives:

$$\left(\frac{\partial H}{\partial P}\right)_T = B - T\frac{\mathrm{d}B}{\mathrm{d}T}, \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$\left(\frac{\partial C_P}{\partial P}\right)_T = -T\frac{\mathrm{d}^2B}{\mathrm{d}T^2}, \quad \ldots\ldots\ldots\ldots \quad (4)$$

which, applied to (**2**), yield the relations:

$$\left(\frac{\partial H}{\partial P}\right)_T = \alpha + \frac{2\beta}{T} + \frac{3\gamma}{T^2} + \frac{4\delta}{T^3}, \quad \ldots\ldots\ldots\ldots \quad (5)$$

$$\left(\frac{\partial C_P}{\partial P}\right)_T = -\frac{2\beta}{T^2} - \frac{6\gamma}{T^3} - \frac{12\delta}{T^4}. \quad \ldots\ldots\ldots\ldots \quad (6)$$

The derivative $(\partial H/\partial P)_T$ has been measured for benzene but not for the other two gases. For benzene, formula (**5**) predicts values in excellent agreement with the experimental data of Gilliland and Lukes (1940, Table 4). This agreement justifies our acceptance of the high-temperature virial coefficients of Gornowski, Amick, and Hixson (1947).

TABLE 4

COMPARISON OF CALCULATED AND OBSERVED ENTHALPIES OF IMPERFECTION FOR BENZENE VAPOUR

Temperature (°K)	$(\partial H/\partial P)_T$ (cal/mole atm)	
	Calc. from (**5**)	Found*
562	—26·19	—27±2
618	—20·53	—19·1±1·5
672	—16·39	—15·3±1·5

* By extrapolating the data of Gilliland and Lukes (1940) to zero pressure.

The effect of pressure on C_P has been measured for *cyclo*hexane at one temperature, and for benzene at five temperatures. The experimental and calculated changes are compared in Table 5. Here the agreement is not quite so good, but the discrepancies could arise from inaccuracies in the rather difficult measurements of specific heats. Unfortunately there is no way of estimating the experimental errors.

IV. Discussion

Apart from their thermodynamic significance, the second virial coefficients can provide valuable information about the interaction forces between the molecules of a gas. If the molecules are non-polar and are either spherical or effectively so by virtue of their rotation, then the interaction energy $\varphi(r)$ between

a pair of isolated molecules depends only on their separation r. In the absence of a rigorous theory of intermolecular forces it is customary to assume that both the attractive and repulsive components of the total potential vary as some inverse power of the separation (Mie 1903), so that $\varphi(r)$ can be written:

$$\begin{aligned}\varphi(r) &= Ar^{-n} - Br^{-m} \\ &= \varepsilon^* \left[\frac{m}{n-m}\left(\frac{r^*}{r}\right)^n - \frac{n}{n-m}\left(\frac{r^*}{r}\right)^m\right], \qquad \text{(7)} \\ n &> m,\end{aligned}$$

where $-\varepsilon^*$ denotes the minimum potential energy corresponding to the separation $r=r^*$. This will be referred to as an (n,m) potential.

TABLE 5

COMPARISON OF CALCULATED AND OBSERVED HEAT CAPACITIES OF IMPERFECTION FOR CYCLOHEXANE AND BENZENE VAPOURS

Gas	Temperature (°K)	$(\partial C_p/\partial P)_T$ (cal/mole deg atm)	
		Calc. from (6)	Found
*cyclo*Hexane	370·9	0·985	0·916*
Benzene	341·6	0·575	0·73†
	371·2	0·444	0·55
	402·3	0·345	0·37
	436·2	0·267	0·24
	471·1	0·208	0·17

* McCullough, Person, and Spitzer (1951).
† Scott *et al.* (1947).

Lennard-Jones (1924) showed that the second virial coefficient of an (n,m) gas is given in classical statistics* by the relation

$$B=\tfrac{2}{3}\pi N r^{*3}\left(\frac{m}{n}\right)^{3/(n-m)} y^{3/(n-m)}\left[\Gamma\left(\frac{n-3}{n}\right)-\frac{3}{n}\sum_{s=1}^{\infty}\Gamma\left(\frac{sm-3}{n}\right)\frac{y^s}{s!}\right], \quad \text{(8)}$$

where

$$y^n=\left(\frac{n}{n-m}\right)^n\left(\frac{n-m}{m}\right)^m\left(-\frac{\varepsilon^*}{kT}\right)^{n-m},$$

and N is Avogadro's number, k is Boltzmann's constant, and Γ denotes the gamma-function.

From an analysis of the virial coefficients of the inert gases Lennard-Jones (1938) concluded that their repulsive and attractive exponents n and m are close to 12 and 6 respectively, and Corner (1948) later showed that this (12,6) potential gives a good representation of the crystal properties of the solidified inert gases. A large number of theories of the properties of fluids have been based on the (12,6)

* The quantal corrections to formula (8) are negligible for the gases discussed in this paper.

potential and it has often been applied, unjustifiably, to quite complex molecules. It should be regarded merely as an empirical relation which holds for very simple molecules.

In an earlier paper (Hamann and Lambert 1954*a*) an attempt was made to estimate n and m for some more complex polyatomic molecules. It was assumed that the molecules were centro-symmetrical and that each atom in a particular molecule interacted separately with each atom in another, according to a (12,6) potential law. For instance the total potential in a system of two tetrahedral molecules $(AB_4)_\alpha$, $(AB_4)_\beta$, was taken as the sum of all the $A_\alpha-A_\beta$, $A_\alpha-B_\beta$, $B_\alpha-A_\beta$, $B_\alpha-B_\beta$ interactions, each of the (12,6) type, and the summed potential was then averaged for all orientations of each molecule. The resulting expression was complicated, but for some typical molecules such as CF_4, OsO_4, SF_6, it could be represented quite well by a (28,7) bireciprocal formula of the type (**7**). The effect of the peripheral atoms is thus to increase both the attractive and repulsive exponents, that is, to steepen the walls of the potential well.

Table 6

INTERMOLECULAR POTENTIAL PARAMETERS DERIVED FROM SECOND VIRIAL COEFFICIENTS

Gas	ε^*/k (°K)			r^* (A)			$d_{\text{calc.}}$ (A)
	Potential :			Potential :			
	(12,6)	(26,6·5)	(28,7)	(12,6)	(26,6·5)	(28,7)	
*cyclo*Propane	210	460	610	6·8	5·2	4·5	5·8
*cyclo*Hexane	(230)†	480	590	(10·1†)	8·0	7·3	7·0
Benzene ..	(240)†	470	570	(9·7†)	7·8	7·2	7·1

† For these gases it was impossible to obtain a good fit between the experimental values of B and the theoretical (12,6) values. This fact confirms the inadequacy of the (12,6) potential.

The cyclic hydrocarbons consist entirely of peripheral groups and we might therefore expect them to show large deviations from the behaviour of the simple (12,6) gases. A deviation of this kind has been observed previously in the surface tension of benzene, which resembles that of a (28,7) fluid rather than a (12,6) one (Hamann and Lambert 1954*b*). Similar deviations are seen when the reduced second virial coefficients B/V_c are plotted against the reduced temperature T/T_c; V_c being the critical volume and T_c the critical temperature. The principle of corresponding states (Pitzer 1939) requires such plots to be coincident for gases whose intermolecular forces have the same general form. Treating the data in Figures 1 and 2 in this way, we find that although the reduced second virial coefficients of benzene and *cyclo*hexane are themselves similar, they are quite different from those of simple monatomic and diatomic gases (taken from Guggenheim 1953). Rowlinson (1954) has found the same kind of deviations in the reduced vapour pressures and reduced surface tensions of the two substances. *cyclo*Propane, however, shows much smaller deviations, as might be expected from its compact structure.

A direct and sensitive test of the validity of a particular interaction potential is to see how well the parameter r^* (the equilibrium separation of two molecules) associated with it, agrees with the known dimensions of the molecules. In doing so we assume that the rotation of each molecule causes it to sweep out a spherical space which is not accessible to other molecules. The diameter d of this region can be calculated from the geometry of the molecule together with the van der Waals radii of its outer atoms (Hamann and Pearse 1952). If the assumed potential is a realistic one then the value of r^* derived from the second virial coefficients should be equal to d. Table 6 lists some values of r^* which we have calculated from the experimental virial coefficients, taking three different sets of exponents in formula (**7**).

It is clear that the (28,7) potential is the most realistic one for *cyclo*hexane and benzene, whereas the potential for *cyclo*propane appears to lie between the (12,6) and (26,6·5) forms.

V. Acknowledgments

We wish to thank Mr. J. A. Lambert for carrying out the summations of formula (**8**) for the (26,6·5) potential.

VI. References

Allen, P. W., Everett, D. H., and Penney, M. F. (1952).—*Proc. Roy. Soc.* A **212** : 149.

Andon, R. J. L., Cox, J. D., Herington, E. F. G., and Martin, J. F. (1957).—*Trans. Faraday Soc.* **53** : 1074.

Bottomley, G. A., Remmington, T. A., and Whytlaw-Gray, R. (1958*a*).—*Proc. Roy. Soc.* A **246** : 504.

Bottomley, G. A., Remmington, T. A., and Whytlaw-Gray, R. (1958*b*).—*Proc. Roy. Soc.* A **246** : 514.

Casado, F. L., Massie, D. S., and Whytlaw-Gray, R. (1951).—*Proc. Roy. Soc.* A **207** : 483.

Corner, J. (1948).—*Trans. Faraday Soc.* **44** : 914.

David, H. G., and Hamann, S. D. (1958).—" Proceedings of Conference on Thermodynamic and Transport Properties of Fluids." p. 74. (Instn. Mech. Engrs. : London.)

Francis, P. G., McGlashan, M. L., Hamann, S. D., and McManamey, W. J. (1952).—*J. Chem. Phys.* **20** : 1341.

Gilliland, E. R., and Lukes, R. V. (1940).—*Industr. Engng. Chem.* **32** : 957.

Gornowski, E. J., Amick, E. H., and Hixson, A. N. (1947).—*Industr. Engng. Chem.* **39** : 1348.

Guggenheim, E. A. (1953).—*Rev. Pure Appl. Chem.* **3** : 1.

Hamann, S. D., and Lambert, J. A. (1954*a*).—*Aust. J. Chem.* **7** : 1.

Hamann, S. D., and Lambert, J. A. (1954*b*).—*Aust. J. Chem.* **7** : 219.

Hamann, S. D., McManamey, W. J., and Pearse, J. F. (1953).—*Trans. Faraday Soc.* **49** : 351.

Hamann, S. D., and Pearse, J. F. (1952).—*Trans. Faraday Soc.* **48** : 101.

Hirschfelder, J. O., Curtiss, C. F., and Bird, R. B. (1954).—" Molecular Theory of Gases and Liquids." p. 131. (Wiley & Sons Inc. : New York.)

Lambert, J. D., Roberts, G. A. H., Rowlinson, J. S., and Wilkinson, V. J. (1949).—*Proc. Roy. Soc.* A **196** : 113.

Lennard-Jones, J. E. (1924).—*Proc. Roy. Soc.* A **106** : 463.

Lennard-Jones, J. E. (1938).—*Physica s'Grav* **4** : 941.

McCullough, J. P., Person, W. B., and Spitzer, R. (1951).—*J. Amer. Chem. Soc.* **73** : 4069.

Mie, G. (1903).—*Ann. Phys. Lpz.* [4] **11** : 657.

Mund, W., Gerbaux, A., and Mornigny, J. (1951).—*Bull. Acad. Belg.* **37** : 706.

Münze, R., and von Weber, V. (1957).—*Naturwissenschaften* **44** : 85.

Onnes, H. K. (1902).—*Proc. Amst. Sci. Acad.* **4** : 125.

Pitzer, K. (1939).—*J. Chem. Phys.* **7** : 583.

ROTINJANZ, L., and NAGORNOW, N. (1934).—*Z. phys. Chem.* A **169** : 20.
ROWLINSON, J. S. (1954).—*Trans. Faraday Soc.* **50** : 647.
SCOTT, D. W., WADDINGTON, G., SMITH, J. C., and HUFFMAN, H. M. (1947).—*J. Chem. Phys.* **15** : 565.
STEURER, E., and WOLF, K. L. (1938).—*Z. phys. Chem.* B **39** : 101.
STULL, D. R. (1947).—*Industr. Engng. Chem.* **39** : 517.
THIESEN, M. (1885).—*Ann. Phys. Chem.* **24** : 467.
WAELBROECK, F. G. (1955).—*J. Chem. Phys.* **23** : 749.
YOUNG, S. (1910).—*Proc. Roy. Dublin Soc.* **12** : 374.

THE "VARIABLE ELECTRONEGATIVITY" METHOD*

II. PYRROLE

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Summary

The properties of the π-electron system in pyrrole have been studied by a "variable electronegativity" self-consistent field molecular-orbital method and the results compared with those obtained by the conventional SCF procedure. The π-electron distribution calculated by the conventional SCF procedure cannot be satisfactorily reconciled with the observed dipole moment, but the distribution calculated by the VESCF method leads to a predicted dipole moment in good agreement with observation. Polarization of σ-bonds makes a negligible contribution to the dipole moment.

Derivation of the coulomb parameters for the simple Hückel molecular-orbital method from the VESCF results is considered and the factors responsible for auxiliary inductive effects are discussed. The π-electron ionization potential and the positions of the lowest excited states of pyrrole have been calculated by the VE method.

I. Introduction

The variable electronegativity (VESCF) method‡ is an elaboration of the conventional SCF method (Roothaan 1951; Pople 1953). In the former the orbital exponent Z_μ for the atomic orbital χ_μ is regarded as a function of the electron density at atom μ and thus various basic integrals, all of which depend upon the values of orbital exponents, become functions of the electron distribution. In the conventional SCF procedure they are assigned constant values corresponding to a uniform electron distribution. Our initial trial of the VESCF method for the π-electrons in formaldehyde suggested that it gives a markedly better estimate of the electron distribution in heterosystems than does the conventional SCF method. The defects of the SCF method are particularly evident if one tries to apply it to π-electron systems such as that in pyrrole because the molecular core now incorporates N^{++} and if the standard procedure (Goeppert-Mayer and Sklar 1938; Pariser and Parr 1953*a*) for evaluating coulomb integrals in terms of valence-state ionization potentials is used, this is equivalent to regarding the nitrogen as vastly more electronegative than the carbons. Consequently little delocalization of electrons from the nitrogen $2p\pi$-orbital results. As will be demonstrated quantitatively below, the dipole

* Brown and Heffernan's (1958) paper is regarded as Part I of this series.

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‡ In Part I of this series the method was termed the "self-consistent electronegativity" (SCE) method. We now feel that the more compact name used here is preferable, especially as the abbreviation SCE is used in another connection by electrochemists.

moment, evaluated from the SCF charge distribution, falls well below the observed value, yet for heterocycles such as pyridine, in which the core nitrogens are of the N^+ type, or for hydrocarbon systems such as azulene, where no heteroatom problem arises, the SCF method overestimates the π-electron contribution to the dipole moment by roughly a factor of two.

We have therefore undertaken a study of pyrrole by the VESCF method to determine how successfully it deals with this type of π-electron system. For comparative purposes a conventional SCF method has also been applied. The present results have bearing on two other aspects of the theoretical treatment of conjugated systems. Firstly, values of the heteroparameters which enter into the simple Hückel MO treatment of pyrrole may be found such that with them the simple MO method reproduces the same π-electron distribution as is obtained by the VESCF method; these parameters might be useful for Hückel MO calculations on large systems containing pyrrolic nitrogen atoms where the VESCF calculations would be impracticable. Secondly, the CIVESCF method (a configuration-interaction treatment amounting to an elaboration of the Pariser-Parr (1953*a*) method for treating ultraviolet spectra) predicted spectroscopic intervals for formaldehyde which did not disagree with the slight experimental information available; this aspect of the VESCF method needs testing on other molecules. Its application to the spectrum of pyrrole is considered here.

II. Method

The main details of the VESCF procedure were set out in Part I of this series (Brown and Heffernan 1958). Of the various types of integral which arise in the method, all of which vary with the orbital exponent Z_μ of each $2p\pi$-orbital appearing in the integrand, it was noticed that only the values of valence-state ionization potentials, I_μ, and monocentric coulomb repulsion integrals* γ_μ are sensitive to Z_μ. The bicentric coulomb repulsion integrals $\gamma_{\mu\nu}$ do not vary appreciably and since they are determined by an empirical interpolation procedure it was decided to reduce the labour of the present calculations by using fixed values for such integrals. It is felt that other uncertainties in the method outweigh small errors which might arise from this simplification.

The variation of I_μ with Z_μ was determined in Part I of this series by quadratic interpolation along the appropriate valence-state isoelectronic series of ions. For example, in the case of a $2p\pi$-orbital for carbon in a V_4, sp^3 state, ionization potentials for C, N^+, and O^{++} in this valence state were taken to be representative for orbitals with $Z_\mu=3\cdot25$, $4\cdot25$, and $5\cdot25$ respectively. However, both the orbital exponent and the nuclear charge vary along this series and in the VESCF method the variation in I_μ caused by variation in Z_μ for constant nuclear charge is required. Now the valence state ionization potentials are used to represent the core attraction of atom μ for an electron in orbital χ_μ and such one-electron

* In previous papers we have used the symbols $(\mu\mu \mid \mu\mu)$ and $(\mu\mu \mid \nu\nu)$ for monocentric and bicentric coulomb repulsion integrals respectively, but have here changed the notation to coincide with the usage of Brickstock and Pople (1954), McWeeny (1956), McWeeny and Peacock (1957), and others.

core-attraction integrals, when evaluated using Slater functions, are proportional* to $Z_\mu Q_\mu$, Q_μ being the valency of the atom, that is, the positive charge on the core when the inner shell electrons are considered to be condensed into the nucleus (thus Q_μ takes the values 4, 5, 6 for C, N^+, and O^{++} respectively). To obtain I_μ for carbon therefore the ionization potentials of C, N^+, and O^{++} were multiplied by 4/4, 4/5, and 4/6 respectively before interpolation, the resultant formula for C (V_4, sp^3) being

$$I(Z_\mu)_C = 0\cdot3605Z_\mu^2 + 9\cdot0923Z_\mu - 21\cdot818 \text{ eV}, \quad \ldots\ldots\ldots \quad (1)$$

while for N^+ (V_4, sp^3) the corresponding expression was

$$I(Z_\mu)_{N^+} = 0\cdot4510Z_\mu^2 + 11\cdot3625Z_\mu - 27\cdot267 \text{ eV}. \quad \ldots\ldots\ldots \quad (2)$$

Two alternative methods of estimating the core coulomb integrals

$$\int \chi_\mu \mathsf{H}^c \chi_\mu d\tau = \alpha_\mu$$

were tried. These methods, *A* and *B* below, amount to different approximations for $(\mu \mid \mathsf{V}_\nu \mid \mu)$. In the expression

$$\alpha_\mu = -I_\mu + \sum_{\nu \neq \mu} (\mu \mid \mathsf{V}_\nu \mid \mu) - \Sigma_\lambda(\lambda : \mu\mu). \quad \ldots\ldots\ldots\ldots \quad (3)$$

The term $(\mu \mid \mathsf{V}_\nu \mid \mu)$ represents the potential energy of an electron in orbital χ_μ arising from the attractive field of the νth atomic core, the first summation being over all conjugated atoms ν, and $(\lambda : \mu\mu)$ is the penetration integral (Pariser and Parr 1953*a*) of orbital μ into the neutral atom λ, the second summation being over all atoms which are not part of the conjugated system (i.e. hydrogen atoms in the case of pyrrole). The core-attraction integral may be written

$$(\mu \mid \mathsf{V}_\nu \mid \mu) = (\mu \mid \mathsf{V}_\nu^\dagger \mid \mu) - \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

where $\mathsf{V}_\nu^\dagger$ represents the potential field of atomic core ν plus a π-electron in χ_ν and is usually equivalent to a neutral atom in its valence state. The first procedure, *A*, consisted in evaluating $(\mu \mid \mathsf{V}_\nu^\dagger \mid \mu)$ theoretically using tables of molecular integrals (Roothaan 1955). The second procedure, *B* (recommended by McWeeny 1956), consisted of neglecting the potential fields of all neutral atoms, so that the second summation in (3) is neglected and we put

$$(\mu \mid \mathsf{V}_\nu \mid \mu) = -X_\nu \gamma_{\mu\nu}, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

X_ν being the charge on core ν (i.e. unity for each carbon atom, two for the secondary nitrogen in pyrrole). It seems likely that procedure *A* overestimates the core attraction and that *B* underestimates it. The VE calculations were performed using both procedures.

* This is true for the major part of the core-attraction term, but the latter also contains a kinetic term proportional to Z_μ^2. Although the virial theorem might be invoked to assign the proportions of the two types of term, the resultant interpolation becomes more cumbersome. The correction to the isoelectronic ionization potentials does not have a great effect on the VESCF calculations and in view of other sources of uncertainty in the calculations the simpler interpolation was considered to be adequate (see also Section III).

The carbon-carbon resonance integrals were derived from the formula given by Pariser and Parr (1953*b*) using the known geometry of pyrrole (Bak *et al.* 1956) which is summarized in Table 1. The carbon-nitrogen resonance integral, β_{CN}, was determined from the ultraviolet spectrum of pyrrole (Pickett *et al.* 1953). The positions of the first few excited states relative to the ground state were found, as a function of β_{CN}, by a configuration interaction treatment and the energy of the longest-wavelength $\pi-\pi^*$ transition (5·876 eV) was used to evaluate β_{CN}. The configuration-interaction treatment is dependent on the form of the molecular orbitals in terms of which configurations are expressed. When a set of orbitals corresponding to a uniform charge distribution of 1·2 π-electrons at each atom was selected, the theoretical value for the spectroscopic interval could not be fitted to the experimental value for any value of β_{CN}, presumably because the selected orbitals were too far from the self-consistent orbitals. However, with a set of orbitals corresponding to a π-electron density of two on the nitrogen and unity on each carbon an initial value $\beta_{CN} = -1·26$ eV

TABLE 1

PYRROLE GEOMETRY

NC_2	1·383 A	NH_2	2·142 A
NC_3	2·229	C_2H_2	1·075
C_2C_3	1·371	C_2H_1	2·120
C_2C_4	2·258	C_2H_3	2·184
C_2C_5	2·251	C_3H_4	2·243
C_3C_4	1·429	C_3H_2	2·231
NH_1	0·993	C_3H_3	1·075

was found. Using this value we applied a conventional SCF treatment to pyrrole to obtain a new set of molecular orbitals. A configuration-interaction treatment based on the new orbitals gave $\beta_{CN} = -2·23$ eV, which is more in keeping with values found for other nitrogen heterocycles (Pariser and Parr 1953*b*; Brown and Heffernan 1957; Dewar and Paoloni 1957). Further iterative determinations of molecular orbitals and values for β_{CN} led to the final value of $-2·30$ eV as being consistent with the final VE orbitals. It was noticeable in the calculations that interaction between the ground state $^1A_1$ wavefunction and excited functions of this symmetry was considerable when the original extreme sets of molecular orbitals were used as basis and that as the calculations converged to SCF orbitals the extent of configuration interaction rapidly decreased, a behaviour used by Lefebvre (1955) as a basis for obtaining SCF functions by a configuration-interaction calculation.

As indicated above, the variation of other integrals, such as penetration integrals, with Z_μ was neglected, the fixed values 3·25 for carbon orbitals and 3·90 for the nitrogen orbital being used for Z_μ in the theoretical evaluation of integrals. The values of integrals which were taken to be insensitive to the π-electron distribution are given in Table 3. Their relative insensitivity to Z_μ can be seen from comparison of values in Table 2 and Table 3. Values for integrals whose dependence upon orbital exponents was included in the computa-

tions are listed in Table 4 for final VE orbitals based on two different values of β_{CN}. The data in Table 4 show how significantly the ionization potentials I_μ and coulomb integrals α_μ depend upon electron density, demonstrating the difference between the SCF method (fixed Z_μ) and the VE method.

TABLE 2

INTEGRALS IN SCF TREATMENT OF PYRROLE (ev)

Based on $Z_C=3 \cdot 25$; $Z_{N^+}=4 \cdot 25$

α_1	—59·202*	(1 : 22)	9·759
α_2	—49·459	(1 : 33)	6·261
α_3	—46·065	(2 : 11)	0·625
		(2 : 33)	0·713
β_{23}	—2·650	(2 : 44)	0·017
β_{34}	—1·905	(2 : 55)	0·017
		(3 : 11)	0·032
γ_1	14·000	(3 : 44)	0·569
γ_2	10·706	(H_1 : 11)	0·910
γ_{12}	7·935	(H_1 : 22)	0·017
γ_{13}	5·948	(H_2 : 11)	0·021
γ_{23}	7·391	(H_2 : 22)	0·623
γ_{24}	5·711	(H_2 : 33)	0·011
γ_{25}	5·723	(H_3 : 22)	0·013
γ_{34}	7·270	(H_3 : 44)	0·011

Other integrals are equivalent to one of the tabulated values

* Calculated assuming the ionization potential of N^+ to be 29·17 eV.

Other details of the computations not given above have been set out previously in Part I of this series (Brown and Heffernan loc. cit.).

TABLE 3

INTEGRALS FOR WHICH CONSTANT VALUES WERE USED IN THE VE METHOD (ev)

γ_{13}	5·886	(H_1 : 11)*	0·872	(2 : 11)*	0·648	(1 : 22)†	9·862
γ_{12}	7·739	(H_2 : 11)*	0·016	(3 : 11)*	0·005	(1 : 33)‡	6·261

For other two-centre integrals, not involving N, values given in Table 2 were used

* In procedure *B* for evaluating core attractions these integrals were taken to be zero.

† In procedure *B* this integral was put equal to γ_{12}.

‡ In procedure *B* this integral was put equal to γ_{13}.

III. THE π-ELECTRON DISTRIBUTION

The π-electron distribution in pyrrole, calculated by several alternative methods, is given in Table 5. Comparison of the second and third row of values in this table shows that the corrected method of interpolating ionization potentials makes only a small difference to the results so that any more elaborate procedure*

* See footnote, p. 321.

is not warranted. Comparison of the third and fourth rows reveals that the calculated charge distribution is insensitive to the value selected for β_{CN} in the VE method and so does not depend critically upon the knowledge of $\pi-\pi^*$ spectroscopic intervals used to estimate β_{CN}. The data of Table 4 also show the insensitivity of the VESCF method to the value of β_{CN}.

The relationship between the conventional SCF procedure and the VESCF procedure may be judged by comparison of the first and third rows of Table 5 (or alternatively of the first and fourth rows because the value $-2\cdot45$ eV for β_{CN} is "spectroscopically consistent" with the SCF method just as the value

TABLE 4

FINAL VALUES (IN eV) FOR INTEGRALS WHICH WERE VARIED WITH Z_μ

	$\beta_{CN}=-2\cdot45$ eV	$\beta_{CN}=-2\cdot30$ eV	
Z_1*	4·025	4·019	
Z_2*	3·195	3·197	
Z_3*	3·243	3·243	
γ_1	13·258	13·239	
γ_2	10·524	10·532	
γ_3	10·681	10·682	
I_1	25·772	25·687	
I_2	10·910	10·938	
I_3	11·455	11·459	
α_1	—55·232	—55·147	—52·725†
α_2	—48·736	—48·764	—45·537†
α_3	—45·918	—45·922	—43·402†
F_{11}	—14·420	—14·344	—12·213†
F_{22}	—9·920	—9·911	—6·419†
F_{33}	—8·208	—8·214	—5·785†

* These entries are pure numbers.

† These values correspond to procedure *B*, all others to procedure *A*.

$-2\cdot30$ eV is "spectroscopically consistent" with the VE method) and of data in Tables 2 and 4. The fact that the monocentric integrals, in particular I_μ, in the SCF method are held at values consistent only with integral electronic charges on atoms leads to considerable localization of π-electrons in pyrrole owing to the intense and invariant field thus assigned to the nitrogen core. However, when the VE procedure is used, more extensive delocalization of π-electrons is found.

The data in rows four and five of Table 5 show that when neutral atom penetrations are neglected the calculated electron densities at the 2- and 3-positions of pyrrole do not differ as much as those obtained when theoretical values of penetrations are included in the calculations. The effect of the penetration integrals is to increase the effective electron-attracting power of the 2-position

at the expense of the 3-position (and also of the nitrogen atom). This point has bearing on the significance of auxiliary inductive parameters used in the Hückel MO method, as is discussed below.

The contrast between the conventional SCF and the VE methods is still more evident when the π-electron density data are used to calculate the dipole moment of pyrrole. For pyrrole there is no lone-pair hybridization contribution

TABLE 5
π-ELECTRON DENSITIES IN PYRROLE

Method	β_{CN} (eV)	P_1	P_2	P_3
SCF	—2·45	1·783	1·120	0·989
VESCF* ..	—2·45	1·591	1·166	1·038
VESCF ..	—2·45	1·640	1·153	1·027
VESCF ..	—2·30	1·658	1·151	1·019
VESCF† ..	—2·30	1·700	1·076	1·074

* Using the variation of I_μ with Z_μ adopted in Part I of this series, instead of equations (**1**) and (**2**).

† Using procedure *B* for core attractions ; in all other cases listed in the table, procedure *A* was used.

to the moment and hydrogen orbital hybridization (Brown and Heffernan 1958) makes a negligible contribution (about 0·04 D). The moments calculated from SCF and VESCF π-electron density data, with the assumption of an unpolarized core, as was made in Part I, are compared with the experimental moment (Wesson 1948) in Table 6.

TABLE 6
DIPOLE MOMENT (IN D) OF PYRROLE

SCF	VESCF			Expt.
0·71	1·77*	1·59†	2·12‡	1·80

* Procedure *A* for core attractions, β_{CN} = —2·45 eV.
† Procedure *A* for core attractions, β_{CN} = —2·30 eV.
‡ Procedure *B* for core attractions, β_{CN} = —2·30 eV.

The SCF value is clearly too low. One can hardly invoke polarization of the σ-bonds to explain the discrepancy because, the σ-moment being in the opposite sense, one would have to assume enormous polarization of the CN σ-bonds, much more than the π-bond polarizations, to account for the observed moment. The VESCF results attribute virtually the whole of the moment to π-electron delocalization, the agreement between observation and theory being reasonably close. The dipole moments calculated using the two alternative procedures, *A* and *B*, for evaluating core-attraction integrals in the VE method fall on either side of the observed moment. This tends to support our suggestion

that the most appropriate values of these integrals probably lie between the extremes obtained by procedures *A* and *B*.

The π-electron densities obtained by any of the procedures listed in Table 5 are in qualitative agreement with the observed chemical reactivity of pyrrole, namely, that it is very susceptible to electrophilic attack, the 2-position being preferentially attacked.

IV. Parameters for Hückel MO Method

When the Hückel MO method is applied to pyrrole it is possible to obtain any required π-electron distribution by choosing suitable values for the electronegativity parameter for nitrogen, h, and the auxiliary inductive parameter for the two neighbouring carbon atoms, h'. The majority of calculations on heterocycles have been performed by a " simple " Hückel method in which resonance integrals between non-neighbouring atoms are neglected, a standard value β^0 is ascribed to the resonance integral of each carbon–carbon bond and

Table 7

HÜCKEL COULOMB PARAMETERS FOR PYRROLE

Position	From VESCF π-Electron Densities of Table 5	
	Row 4	Row 5
1	1·88	2·00
2	0·42	0·18
3	0	0

an empirical value, usually β^0, is selected for the resonance integral of the carbon–nitrogen bond. The values of h and h' which reproduce the VE π-electron densities of pyrrole when used in the " simple " Hückel method are given in Table 7. These are not very far from the values originally used by Wheland and Pauling (1935) in the first Hückel MO treatment of pyrrole. The values of parameters used in their work were selected primarily to provide a π-electron distribution in agreement with the observed orientation of electrophilic substitution.

Two points call for comment. Firstly, the parameter values for secondary nitrogen in a five-membered ring deduced from the present VE study of pyrrole are vastly different from that which, from similar studies, seems appropriate for tertiary nitrogen in a six-membered ring (McWeeny and Peacock 1957; Mataga and Nishimoto 1957; Brown and Heffernan 1959), namely, about 0·2 for h and a much smaller value for h'. However, it has been common practice in Hückel MO calculations to use the same values of parameters for both types of nitrogen.

Secondly, an appreciable auxiliary inductive parameter is called for to obtain the VESCF charge distribution. Auxiliary inductive parameters were introduced by Wheland and Pauling (1935) because it was not possible to account,

in terms of π-electron densities, for the preferential attack of the 2-position in pyrrole by electrophils unless such a parameter is used. Since it was not demonstrated to be necessary to include such parameters to account for any other chemical or physical property of pyrrole or other conjugated systems, and it was shown (Brown 1955) that 2-substitution in pyrrole could be explained using electrophilic localization energies and no auxiliary parameter, the principle of simplicity could be invoked as grounds for omitting this additional coulomb parameter in the Hückel MO method. When more recently (Brown 1958) evidence accumulated that for all but the most unreactive heterocyclic systems the orientation of electrophilic substitution follows the π-electron distribution rather than localization energies, the chemical properties once again became admissible evidence for the need to include auxiliary inductive parameters. The present VESCF calculations now provide independent evidence supporting the use of these additional parameters.

A comparison of the Hückel MO parameters required to produce the VE electron distributions obtained when neutral-atom penetration integrals are included and neglected respectively (columns two and three of Table 7) shows that the need for an auxiliary inductive parameter may be attributed primarily to the influence of neutral-atom potentials upon the VE coulomb integrals of the atoms (see for example Table 4). The penetrations tend to make the 2-carbon atom more electronegative than the 3-atom. The optimum values for the Hückel MO parameters are probably intermediate between those listed in columns two and three of Table 7. The values $h=2$, $h'=1/4$ (originally used by Wheland and Pauling) still seem to be appropriate for a pyrrole-type secondary nitrogen.

The present investigations indicate that the cause of the " auxiliary inductive effect " is *not* the production of a small positive charge at the 2-carbon atomic core owing to polarization of the CN σ-bond because the study of the dipole moment (above) shows that σ-polarization is negligible. The presence of a heteroatom in the conjugated system produces a core field at adjacent carbon atoms differing from that at carbon atoms in a carbon-atom environment even when the σ-bonds are purely covalent.

V. $\pi-\pi^*$ Transitions of Pyrrole

The configuration-interaction treatment of pyrrole used to estimate β_{CN} from the transition energy of the longest wavelength $\pi-\pi^*$ spectral transition may also be used to estimate the positions of other low-lying excited states of pyrrole. The results, based on the final VESCF orbitals, are compared with the observed spectrum in Figure 1, results for the alternative treatments of core attractions being designated in the diagram as A and B. The results suggest that the lowest excited state, $5\cdot87$ eV above the ground state, is $^1B_1$. The next state is observed at $6\cdot7$ eV above the ground state and it seems likely that this is of type $^1A_1$. The CIVESCF calculations predict a state of this symmetry at a somewhat lower energy than this, but it seems to be characteristic of SCFCI calculations that the position of the second excited singlet state is predicted to be at lower energies than is observed (compare the results for various six-membered ring heterocycles by McWeeny and Peacock (1957)). A third level is predicted

at 7·2 to 7·3 eV above the ground state in good agreement with an observed level of this energy. Again it seems to be characteristic of calculations of this type that the position predicted for the third excited singlet state falls near the observed position. The alternative methods of treating core-attraction integrals lead to very similar predictions of positions of excited states.

The present treatment predicts two low-lying triplet states for pyrrole, but no such states have been observed experimentally yet.

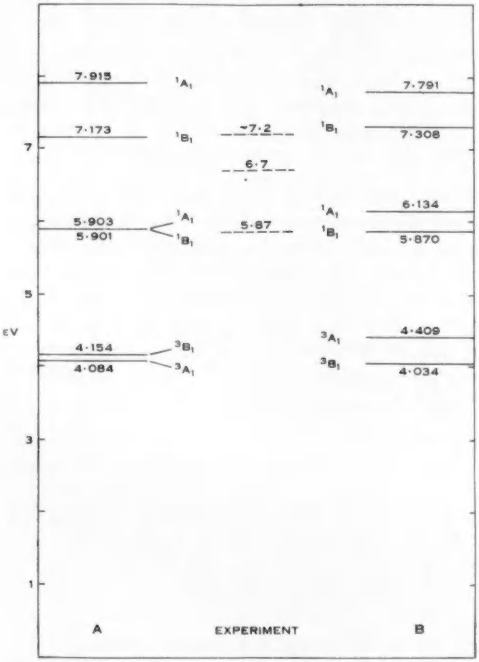

Fig. 1.—Lower excited states of pyrrole, calculated by the VE method using procedure *A* and *B* for core-attraction integrals.

VI. Ionization Potential

Calculated values of π-electron ionization potentials are strongly influenced by the various integral approximations employed (Parr and Pariser 1955). However, differences in ionization potentials of two molecules can be predicted more reliably, this method having been used with reasonable success for example for six-membered ring nitrogen heterocycles using benzene as the reference molecule (Mataga and Nishimoto 1957). In the case of pyrrole, when the ionization potential of benzene (9·24 eV) was used as reference point, the ionization potential was found by the VE method to be 8·72 eV (method *A*) or 7·88 eV

(method *B*). The conventional SCF method gave an ionization potential of 9·10 eV. The experimental value for pyrrole is 8·90 eV (Platt and Klevens 1944).

VII. Acknowledgments

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VIII. References

Bak, B., Christensen, D., Hansen, L., and Rastrup-Andersen, J. (1956).—*J. Chem. Phys.* **24**: 720.

Brickstock, A., and Pople, J. A. (1954).—*Trans. Faraday Soc.* **50**: 901.

Brown, R. D. (1955).—*Aust. J. Chem.* **8**: 100.

Brown, R. D. (1958).—"Current Trends in Heterocyclic Chemistry." p. 13. (Butterworths Scientific Publications: London.)

Brown, R. D., and Heffernan, M. L. (1957).—*Aust. J. Chem.* **10**: 211.

Brown, R. D., and Heffernan, M. L. (1958).—*Trans. Faraday Soc.* **54**: 757.

Dewar, M. J. S., and Paoloni, L. (1957).—*Trans. Faraday Soc.* **53**: 261.

Goeppert-Mayer, M., and Sklar, A. L. (1938).—*J. Chem. Phys.* **6**: 645.

Lefebvre, R. (1955).—*C.R. Acad. Sci. Paris* **240**: 1094.

McWeeny, R. (1956).—*Proc. Roy. Soc.* A **237**: 355.

McWeeny, R., and Peacock, T. E. (1957).—*Proc. Phys. Soc.* A **70**: 41.

Mataga, N., and Nishimoto, K. (1957).—*Z. phys. Chem. N.F.* **13**: 140.

Pariser, R., and Parr, R. G. (1953*a*).—*J. Chem. Phys.* **21**: 466.

Pariser, R., and Parr, R. G. (1953*b*).—*J. Chem. Phys.* **21**: 767.

Parr, R. G., and Pariser, R. (1955).—*J. Chem. Phys.* **23**: 711.

Pickett, L. W., Corning, M. E., Wieder, G. M., Semenow, D. A., and Brickley, J. M. (1953).—*J. Amer. Chem. Soc.* **75**: 1618.

Platt, J. R., and Klevens, H. B. (1944).—*Rev. Mod. Phys.* **16**: 182.

Pople, J. A. (1953).—*Trans. Faraday Soc.* **49**: 1375.

Roothaan, C. C. J. (1951).—*Rev. Mod. Phys.* **23**: 69.

Roothaan, C. C. J. (1955).—"Tables of Two-Centre Coulomb Integrals between 1*s*, 2*s*, and 2*p* Orbitals." (O.N.R. Rep., Univ. Chicago.)

Wesson, L. G. (1948).—"Tables of Electric Dipole Moments." (Technology Press: M.I.T. Cambridge, Mass.)

Wheland, G. W., and Pauling, L. (1935).—*J. Amer. Chem. Soc.* **57**: 2086.

THE "VARIABLE ELECTRONEGATIVITY" METHOD

III. THE PYRROLE ANION AND ELECTRONEGATIVITY REVERSAL

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Summary

The π-electron distribution in the pyrrole anion has been evaluated by the VESCF method using two alternative methods of estimating core attraction terms. The results indicate that the π-electron distribution around the conjugated system is very nearly uniform, supporting previous speculations that the relative attracting powers of tertiary nitrogen and carbon reverse when their π-electron densities exceed a certain value, suspected to be not too much greater than unity. The present calculations indicate that the critical π-electron density is around $1 \cdot 2$.

The VESCF estimates of ionization potentials of the pyrrole anion are also reported.

I. Introduction

The interpretation of the chemistry of five-membered ring heterocycles in terms of π-electron distributions or other theoretical concepts has proved more complex than for six-membered ring heterocycles. Among the concepts that have been introduced is that of electronegativity reversal—a heteroatom which is normally more electronegative than carbon may become less electronegative than carbon atoms in the same molecule if the level of charge densities around the molecule becomes sufficiently high (Bassett and Brown 1954; Brown 1955; Bassett, Brown, and Penfold 1956). Hitherto the evidence supporting the use of this concept in conjugated systems has been indirect, depending largely on an observed change in orientation of electrophilic substitution in glyoxaline in different environments and the assumption that this may be correlated with π-electron densities computed by the Hückel MO method. Evidence is accumulating (Brown and Heffernan 1958, 1959, and further unpublished results) that the VESCF method yields reliable estimates of the π-electron distribution in heterocyclic systems. It thus offers the possibility of a more direct theoretical test of the electronegativity reversal hypothesis than does the Hückel MO method since it is free from some of the ambiguities (e.g. values for coulomb parameters) present in the latter. In fact it is probably best to regard the Hückel method as a convenient calculus for roughly reproducing VESCF results by use of empirically determined parameters. We report here some VE calculations on the pyrrole anion (formed when a proton is lost from the secondary nitrogen atom in pyrrole) designed primarily to throw light on electronegativity reversal.

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II. Method

The details of the VE method have been set out in Parts I and II of this series (Brown and Heffernan 1958, 1959) and will not be repeated here. The geometry of the pyrrole anion has not been determined experimentally ; it was taken to be the same as in pyrrole (I) itself (see Part II). The ultraviolet spectrum also has not been observed previously but it appears to be very similar to that of pyrrole* and so this was assumed for the evaluation of β_{CN} by a CIVESCF calculation. The value obtained was $\beta_{CN}=-2 \cdot 83$ eV. Since this value is somewhat uncertain, VE calculations were also done using another value, $\beta_{CN}=-2 \cdot 30$ eV (the value used for pyrrole in Part II), to determine to what extent the results depend upon the selected value of this resonance integral.

(I)

Table 1

values (in eV) of VESCF quantities

	$\beta_{CN}=-2 \cdot 83$ eV		$\beta_{CN}=-2 \cdot 30$ eV	
	A*	B*	A*	B*
Z_1†	3·840	3·813	3·831	3·800
Z_2†	3·179	3·192	3·180	3·195
Z_3†	3·177	3·177	3·180	3·180
γ_1	12·647	12·560	12·619	12·517
γ_2	10·471	10·514	10·474	10·523
γ_3	10·463	10·464	10·474	10·476
I_1	13·304	12·930	13·183	12·744
I_2	10·727	10·875	10·739	10·908
I_3	10·701	10·703	10·738	10·746
α_1	—41·892	—40·180	—41·771	—39·994
α_2	—40·162	—37·439	—40·174	—37·472
α_3	—39·420	—36·961	—39·457	—37·004

* These refer to the alternative procedures A and B for estimating core attractions described in Part II of this series.

† These are pure numbers.

In addition the two alternative treatments, designated A and B in Part II, for core-attraction integrals were tried.

Values of integrals whose variation with Z_μ was included in the VE calculations are listed in Table 1. Values of most other integrals have been given in Part II, the only additional ones being $(1:22)=1 \cdot 488$ eV and $(1:33)=0 \cdot 517$ eV

* We attempted to observe the longest wavelength absorption band of the anion by determining the absorption of solutions of potassium pyrrole in purified tetrahydrofuran in a Uvispek spectrophotometer. Appreciable absorption commenced only near the short wavelength limit (c. 2100 Å) of the instrument and although no maximum could be reached, the onset of absorption seemed to be very similar to that of pyrrole.

in place of the values given in Part II, the smaller values entering here because of the different nitrogen core present in the pyrrole anion (the nitrogen core charge is +1 instead of the pyrrole value of +2).

III. π-ELECTRON DENSITIES

The results of the VE calculations of π-electron densities, P, are shown in Table 2. The results are seen to be insensitive to the value adopted for the CN resonance integral. Procedures A and B for the core attractions give only

TABLE 2

π-ELECTRON DENSITIES IN THE PYRROLE ANION

Core-Attraction Method	β_{CN} (eV)	P_1	P_2	P_3
A	—2·83	1·175	1·203	1·210
	—2·30	1·199	1·199	1·202
B	—2·83	1·251	1·166	1·209
	—2·30	1·287	1·158	1·199

slightly different charge distributions, in the first case the nitrogen charge being just lower than the charge on the carbons and the reverse applying for procedure B. At present we are unable to decide which of the two treatments of core attractions is the more appropriate; the optimum treatment is doubtless somewhere intermediate between procedures A and B. Thus within our present uncertainty we

TABLE 3

π-ELECTRON IONIZATION POTENTIALS

Core-Attraction Method	β_{CN} (eV)	Ionization Potential (eV)	
		First	Second
A	—2·83	5·57	6·41
	—2·30	5·35	6·43
B	—2·83	3·35	3·97
	—2·30	3·15	3·99

may conclude that in the pyrrole anion the five atoms of the conjugated system share the π-electrons equally and in this sense are of equal electronegativity. In pyridine (Brown and Heffernan 1957; further results in course of publication) the tertiary* nitrogen is appreciably more electronegative than the carbons so

* In the present context this must be read to mean nitrogen of core charge +1 as in pyridine or the pyrrole anion, in contrast to "secondary" nitrogen, meaning of core charge +2, as in pyrrole or the pyridinium cation.

that as the general level of π-electron densities increases from $1 \cdot 0$ to $1 \cdot 2$ the electronegativity of tertiary nitrogen relative to carbon is reduced until the two are equal; the electronegativity of tertiary nitrogen is on the brink of reversing relative to carbon when their π-electron densities are $1 \cdot 2$. Here then is direct theoretical evidence that the concept of electronegativity reversal for tertiary nitrogen in a five-membered ring is a physically reasonable one and it would appear that the critical electron density for reversal is around $1 \cdot 2$.

IV. Ionization Potentials

The values obtained for the π-electron ionization potentials by the various VESCF calculations described here are listed in Table 3. For comparison, the values obtained for the first ionization potential of benzene using procedures A and B for treating core attractions are $14 \cdot 64$ and $12 \cdot 66$ eV respectively, the experimental value being $9 \cdot 24$ eV. The ionization potential of the n-electrons occupying the nitrogen sp^2-hybrid orbital may be estimated as has been described for formaldehyde (Brown and Heffernan 1958), taking the π-electron density on the nitrogen to be $1 \cdot 2$, as $14 \cdot 0$ eV. It seems likely that all of the estimates of ionization potentials are too great by perhaps several eV, as shown by the benzene values quoted above, and as was found for the lone-pair electrons in formaldehyde. As Lykos and Parr (1956) and Coulson (1958) have mentioned, the error probably comes mainly from neglecting the electronic relaxation which will occur in the residual system left after the ionization. As the electron is lost by ionization, the remaining electrons will simultaneously " relax " into more stable configurations so that the energy required for ionization will be smaller than would be the case if such relaxation could not occur. In spite of this error in our estimates of ionization potentials, the difference between the values for the first π-electron and the n-electrons is great enough to leave no doubt that the former is considerably lower than the latter in the pyrrole anion.

The data in Table 3 show that the estimated values of ionization potentials are insensitive to the value selected for β_{CN}. The values of ionization potentials obtained with procedure A for core attractions are larger than those obtained with procedure B. This is to be expected because procedure A always leads to larger values for core attractions than does procedure B. The general " tightness of binding " of π-electrons is therefore greater when method A is used than when method B is used.

V. Acknowledgments

Much of the computation described above was done on a desk calculating machine which was purchased with funds generously provided by Imperial Chemical Industries of Australia and New Zealand Ltd. The Melbourne University computer CSIRAC was used for solving secular equations, using a programme designed by Mr. A. Jones. One of us (M.L.H.) gratefully acknowledges the award of a Studentship by the Commonwealth Scientific and Industrial Research Organization.

VI. References

Bassett, I. M., and Brown, R. D. (1954).—*J. Chem. Soc.* **1954** : 2701.

Bassett, I. M., Brown, R. D., and Penfold, A. (1956).—*Chem. & Ind.* **1956** : 892.

Brown, R. D. (1955).—*Aust. J. Chem.* **8** : 100.

Brown, R. D., and Heffernan, M. L. (1957).—*Aust. J. Chem.* **10** : 211.

Brown, R. D., and Heffernan, M. L. (1958).—*Trans. Faraday Soc.* **54** : 757.

Coulson, C. A. (1958).—Quantum Chemistry Group, Mathematical Institute, Oxford University. Progr. Rep. 1957–58. p. 2.

Lykos, P. G., and Parr, R. G. (1956).—*J. Chem. Phys.* **24** : 1166.

ALTERNATING CURRENT POLAROGRAPHY: EVALUATION OF THE ACCURACY OF THE DATA AND CALCULATION PROCEDURES

By H. H. BAUER* and P. J. ELVING†

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Summary

The accuracy of the data obtained by A.C. polarography when measuring electrode parameters and in analytical work is discussed in a critical review of the actual measurements and calculations involved. The importance of correcting for the series resistances and of using equations which take into account the phase differences between the faradaic and capacitative currents is demonstrated. Other subjects discussed include the nature of the dependence of the peak alternating current on depolarizer concentration, effect of adsorption of depolarizer on the electrode, and simplified procedure for quantitative analysis.

I. INTRODUCTION

Alternating current polarography has been used to test the reversibility of redox processes, to measure fundamental parameters of the electrochemical process, to study adsorption processes, and to perform quantitative analyses. These uses have at times been discussed in a fragmentary way from the viewpoint of the effect of experimental conditions on the precision and validity of the data obtained.

The present study is intended to assay critically the data given by A.C. polarography when applied under the conditions customarily used, as well as when applied under optimum conditions. It is thus hoped that a sound basis will be available for evaluating A.C. polarographic results. The discussion will centre around the procedures used for measurement of the heterogeneous rate constant, since this situation affords the possibility for the most penetrating analysis of the technique.

II. MEASUREMENT OF THE HETEROGENEOUS RATE CONSTANT

The A.C. polarographic technique developed for measurement of the heterogeneous rate constant, k, and the A.C. efficiency, ρ, has been fully set out in a previous paper (Bauer and Elving 1958). Possible errors in the results can arise in the following ways:

(i) Experimental errors in determining the directly measured variables R'_C (cell impedance using only supporting electrolyte) and R'_T (cell impedance in the presence of the redox system).

(ii) Errors produced if the calculated series resistance, R_X, is different from the actual value.

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(iii) Errors due to the indirect, trial and error, methods of calculation necessary in obtaining k and ρ values.

For determination of k and ρ, R'_C and R'_T are measured over a wide frequency range and the resulting values for the faradaic impedance, R_F, are fitted to the theoretical equation by the method of least squares (Bauer and Elving 1958). This procedure minimizes the effect of the experimental error in the determination of single cell-impedance values at any one frequency.

However, the same value of R_X is used at all frequencies to calculate from the cell impedances the corresponding interface impedances.

$$R_C = R'_C - R_X, \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$R_T = R'_T - R_X. \quad \ldots\ldots\ldots\ldots \quad (2)$$

Consequently, an error in the value of R_X used produces a systematic error in all of the interface impedance values. The effect of such an error on the calculated values of k and ρ will be considered in the following paragraphs.

Use of a general equation relating R_X to the calculated k and ρ values is not feasible; any such equation is so complicated that it needs to be solved numerically. The same information can be obtained more conveniently by carrying out typical calculations on a model system.

The following numerical example will be used in this and subsequent sections: $n=2$, $D=10^{-5}$ cm^2 sec^{-1}, $C=1$mM, $T=300$ °K, $A=2\cdot5\times10^{-2}$ cm^2, $f_M=5200$ c/s (corresponding to $k=0\cdot202$ cm sec^{-1}), $R_C=100\ \Omega$ at a frequency of 1000 c/s. These values correspond quite closely to those found in a previous investigation (Bauer and Elving 1958) of the cadmium system in $0\cdot5$M hydrochloric acid.

The cell impedances which would be observed in a study of such a system can be calculated from the values given. The trial and error method of calculation previously described can now be applied to the calculated cell impedances, assuming them to have been experimentally obtained; the results so obtained will indicate the reliability of this method of calculation. The values of ρ ($1\cdot015\pm0\cdot025$; actual value$=1\cdot000$) and f_M (5000 ± 250 c/s; actual value$=5200$ c/s) calculated show the method to be adequate.

To determine the effect of an error in the series resistance correction used in this hypothetical experiment, the cell impedance values are each increased by an amount representing the supposed error in the series resistance and computing the resultant k and ρ values. The results (Table 1) indicate that if k is to be determined to within 10 per cent., then the series resistance correction must be accurate to about 1 Ω in the present case. The total series resistance, then, should not be greater than about 10 Ω, assuming that it can be measured with an accuracy of about 10 per cent.

Estimation of Probable Error in Measurement of k.—The considerations discussed can be used to indicate the accuracy achieved with any system. The percentage error in the calculated value of f_M in the example used turns out to be roughly the same as the error in R'_T at the highest frequency; the percentage error in k will be half of this, since k is proportional to the square root of f_M. This way of estimating the probable error in k appears to be quite generally

valid since the optimum conditions for measurement by A.C. polarography are quite similar for all systems. Although measurements are made over the widest possible frequency range, it has been found that the A.C. wave can no longer be observed when the frequency becomes greater than about $2f_M$. Variation of the capacitative impedance, which depends on the background electrolyte and on the potential at which the redox system displays its maximum faradaic admittance, will not invalidate the general conclusion, since at the highest frequency used, R'_T becomes experimentally indistinguishable from R'_C, and this is the impedance value used to estimate the accuracy of the measurement of k. Consequently, it is immediately apparent from the approximate value of R_T at the highest frequency how accurately one needs to know R_X in order to obtain a value for k with a specified reliability.

TABLE 1

EFFECT OF AN ERROR IN R_X ON THE MEASURED VALUES OF f_M, k, AND ρ

	f_M (c/s)	ρ	k (cm sec^{-1})	Error in ρ (%)	Error in k (%)
Actual values	5200	1·00	0·202		
Calculated values (R_X correct)	5000	1·01$_5$	0·198	2	2
Calculated values (R_X in error by 1 Ω) ..	4500	0·93	0·188	7	7
Calculated values (R_X in error by 2 Ω) ..	3750	0·91$_5$	0·172	9	15

Other optimum conditions for measurement of k are the use of relatively high concentrations of depolarizer in order to make R_T as different from R_C as possible, but on the other hand, concentrations much greater than millimolar should not be used, so that the experimental conditions may remain typically polarographic. The capacitative impedance of the double layer should be as high as possible; on the other hand, the background electrolyte concentration should also be high in order to make the solution resistance small. Similarly, the resistance of the dropping mercury electrode should be kept to a minimum (Loveland and Elving 1952; Randles and Somerton 1952; Bauer and Elving 1958).

Under the optimum conditions outlined, ρ and k values can be obtained, which are reproducible at worst to 10 per cent. and usually to within 5 per cent.*

III. Measurement of the Heterogeneous Rate Constant without Correction for Series Resistances and Phase Differences

In earlier work with A.C. polarography, the importance of making adequate corrections for the series resistances was not fully realized. For example, the first test of the theoretical equation describing the frequency dependence of the

* Unknown sources of error, such as surface contamination which is always a possibility in such work, cannot be included in this estimation.

A.C. wave (Breyer, Bauer, and Hacobian 1955) was made under conditions where the series resistances were of the order of 110 Ω; it was indicated that the resulting values of k could be regarded as having only relative, not absolute, significance. In addition, the faradaic admittance, R_F (or the faradaic alternating current, Δi), was usually calculated not vectorially taking into account the phase differences, but from a scalar equation which assumed the faradaic and capacity currents to be in phase at all frequencies.*

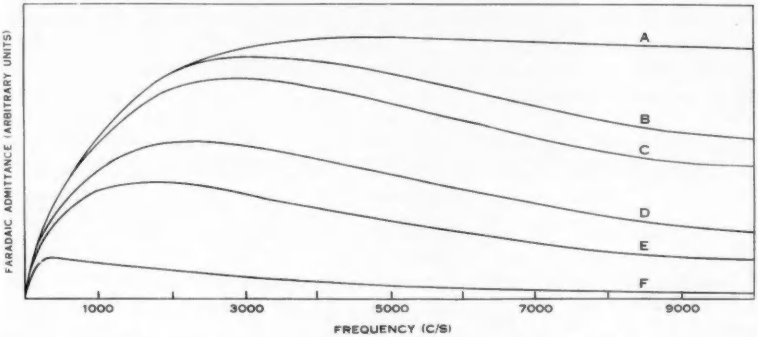

Fig. 1.—Calculated frequency-dependence of the faradaic admittance; scalar calculation neglecting series resistances. *A*, actual value; *B* to *F*, calculated for neglected series resistances R_X of values 0, 1, 5, 10, and 50 Ω respectively.

Figure 1 summarizes numerical calculations made for the previously defined model system to determine the errors introduced when a scalar calculation is made instead of a vectorial one and the further effect of neglecting the series resistances. The results show that even when series resistances are absent, use of the scalar calculation gives quite incorrect results at higher frequencies. In the present case the measured value of f_M under these conditions (3200 c/s) differs greatly from the actual value (5200 c/s): the measured value of k is in error by nearly 30 per cent. (Table 2).

TABLE 2

EFFECT ON THE OBSERVED VALUE OF f_M OF NEGLECTING R_X AND NOT ALLOWING FOR PHASE DIFFERENCES BY VECTORIAL CALCULATION

Actual value of f_M is 5200 c/s

R_X (Ω)	0	1	2	3	5	10	20	30	50	100
f_M (c/s)	3200	2950	2750	2570	2300	1850	1250	915	550	245

* An attempt was made (Breyer, Bauer, and Hacobian 1955) to perform a vectorial calculation by assuming a constant phase difference of 45° between the faradaic current and the capacity current at all frequencies (cf. also Delahay and Adams 1952; Delahay 1954). However, since the theory shows that the phase angle changes with frequency, calculations based on a fixed phase difference are in principle not much better than purely scalar calculations.

When, in addition, a finite series resistance is present, for which correction is not made, the calculated results become increasingly erroneous (Table 2 ; cf. Kalyanasundarum 1951).*

IV. Use of the Scalar Calculation for Determining the Dependence of the Peak Alternating Current on Depolarizer Concentration

When observations are made at frequencies sufficiently low that R_C becomes much greater than R_T, the values of R_F calculated in the scalar way differ only negligibly from those calculated vectorially (Fig. 1 ; cf. curves A and B).

For the sake of simplicity, it will be assumed in the following discussion that experiments aimed at measuring the concentration dependence of the faradaic admittance are being made under conditions where the scalar calculation is sufficiently accurate. The effect of neglected series resistances on the observed concentration dependence will now be investigated.†

Since it has been postulated that the scalar calculation can be used here,

$$\frac{1}{R_F}=\frac{1}{R_T}-\frac{1}{R_C}, \qquad (3)$$

and, in the presence of series resistances R_X,

$$\frac{1}{R_F'}=\frac{1}{R_T+R_X}-\frac{1}{R_C+R_X}; \qquad (4)$$

eliminating R_T,

$$\frac{1}{R_F'}=\frac{R_C^2}{R_CR_X(R_C+R_X)+R_F(R_C+R_X)^2}. \qquad (5)$$

If the actual concentration dependence of the faradaic admittance is linear, then

$$1/R_F=KC; \qquad (6)$$

combining equations (5) and (6) gives

$$\frac{1}{R_F'}=\frac{KCR_C^2}{(R_C+R_X)^2+KCR_CR_X(R_C+R_X)}. \qquad (7)$$

Equation (7) has the form of a Langmuir isotherm

$$1/R_F'=abC/(1+bC), \qquad (8)$$

* In Section III, the effects of series resistances of magnitude up to 100 Ω have been calculated, since such high resistances were present in previous attempts at measuring k (Breyer, Bauer, and Hacobian 1955). In Section II, it was not considered necessary to examine the effects of series resistance errors greater than 2 Ω, since the aim there was to indicate the accuracy achievable with optimum technique ; errors greater than 2 Ω in determining the series resistance correction are not considered likely.

† It has been pointed out (Breyer, Gutmann, and Hacobian 1950) that the observed dependence should be linear for simple redox processes which do not involve adsorption, but non-linear if large series resistances are present. However, the latter effect has not previously been discussed in a quantitative way.

with

$$a = R_C / R_X (R_C + R_X), \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

and

$$b = K R_C R_X / (R_C + R_X). \quad \ldots\ldots\ldots\ldots\ldots \quad (10)$$

Equation (**8**) is shown in graphical form in Figure 2. The parameter a is equal to the maximum faradaic admittance which can be observed at the highest concentrations (as $C \rightarrow \infty$, $1/R'_F \rightarrow a$) ; the parameter b provides a measure of the concentration range, where equation (**8**) is markedly non-linear (as $R_X \rightarrow 0$, $b \rightarrow 0$, i.e. there is no observable non-linearity if series resistances are absent or have been properly compensated) ; b is equal to the reciprocal of that concentration, where the observed faradaic admittance is half of its maximum value.

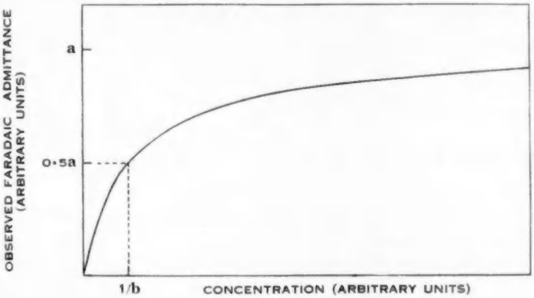

Fig. 2.—Concentration-dependence of the faradaic admittance in the form of a Langmuir isotherm. Significance of parameters a and b.

Effect of Possible Adsorption.—The present discussion is pertinent to the A.C. polarographic behaviour of organic compounds (Breyer, Bauer, and Hacobian 1954). In all cases studied, the concentration dependence of the faradaic admittance had the form of equation (**8**) (in a few cases the curve was more complicated in some details, see Bauer 1955). In several cases a faradaic admittance could be observed at concentrations considerably lower than those which usually yield A.C. polarographic waves, for example, at 10^{-7}M chloroanilic acid (Breyer and Bauer 1955). These observations were explained by postulating adsorption of the organic compound at the surface of the dropping mercury electrode, making the surface concentration, which determines the faradaic admittance, greater than it would be for the unadsorbed substance.

Equations (**7**) to (**10**) show that the concentration dependence of the faradaic admittance may follow a Langmuir isotherm if series resistances have been neglected or incorrectly determined even if the electroactive species are not adsorbed. Consequently, systems which show a concentration dependence similar to that of equation (**8**) cannot safely be classed as processes where the electroactive species are adsorbed, unless the faradaic admittance can be shown to be anomalously high at low concentrations.

It is apparent that even if the actual concentration dependence is non-linear, the parameters a and b obtained will be in error if the effect of series resistances has been neglected. Thus, in a case where the faradaic admittance follows the relation

$$1/R_F = A'B'C/(1+B'C), \quad \ldots \quad (\mathbf{11})$$

rather than equation (**6**), the observed results will follow the equation

$$\frac{1}{R'_F} = \frac{A'B'CR_C^2}{(R_C+R_X)^2+B'C(R_C+R_X)(R_C+R_X+A'R_CR_X)} \quad \ldots \quad (\mathbf{12})$$

obtained by substituting equation (**11**) into (**5**).

Equation (**12**) also has the form of the Langmuir isotherm, but the observed parameters a and b (eqn. (**8**)) are now given by

$$a = A'R_C^2/(R_C+R_X)(R_C+R_X+A'R_CR_X), \quad \ldots \quad (\mathbf{13})$$
$$b = B'(R_C+R_X+A'R_CR_X)/(R_C+R_X), \quad \ldots \quad (\mathbf{14})$$

rather than by equations (**9**) and (**10**). It is readily seen that for $R_X=0$, $a=A'$, and $b=B'$.

V. Use of Alternating Current Polarography as a Technique

The previous sections have been concerned largely with the accuracy obtainable in measuring the heterogeneous rate constant and, in particular, with the effect of neglecting the series resistances. It is evident from this discussion that, to obtain reliable values of k and to investigate such effects as the non-linear concentration dependence of the faradaic admittance, it is quite essential to use a technique in which the series resistances are kept very low (use of special capillaries) and in which the unavoidable minimum resistances which persist (of the order of 10 Ω) are carefully measured and the necessary corrections made. A suitable technique has been fully described (Bauer and Elving 1958).

Fortunately, for routine analytical work such an elaborate and rather laborious technique is not necessary. It is quite feasible to use a conventional capillary and a fixed applied alternating potential, and to measure the faradaic alternating current by a simple scalar equation. If a condenser-pool reference electrode (Breyer, Gutmann, and Hacobian 1951) and a reasonably small (50 or 100 Ω) voltage-dropping resistor to provide the input for the current-measuring device are used, the total neglected series resistances will be 200 Ω. The non-linearity induced in the peak current *v.* concentration relation under such conditions will not be great enough to interfere with accurate analytical estimation by means of a previously prepared calibration curve.

VI. Conclusions

In determining the heterogeneous rate constant, k, by A.C. polarography, failure to correct properly for series resistances and phase differences leads to completely erroneous results.

Bauer and Elving's (1958) technique should permit estimation of k to about 10 per cent. for k values in the range of $0 \cdot 02$ to $0 \cdot 20$ cm sec^{-1}.

Neglect of series resistances can produce non-linear current *v.* concentration relations. Such relations have been taken as indicating the presence of adsorption processes; this interpretation is dubious unless it is also shown that the current response is abnormally high at low concentrations.

For routine analytical work, a much simpler technique can be used which does not require special capillaries nor laborious calculations.

VII. Acknowledgments

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VIII. References

Bauer, H. H. (1955).—Ph.D. Thesis, University of Sydney.
Bauer, H. H., and Elving, P. J. (1958).—*Analyt. Chem.* **30**: 334.
Breyer, B., and Bauer, H. H. (1955).—*Aust. J. Chem.* **8**: 472.
Breyer, B., Bauer, H. H., and Hacobian, S. (1954).—*Aust. J. Chem.* **7**: 305.
Breyer, B., Bauer, H. H., and Hacobian, S. (1955).—*Aust. J. Chem.* **8**: 322.
Breyer, B., Gutmann, F., and Hacobian, S. (1950).—*Aust. J. Sci. Res.* A **3**: 567.
Breyer, B., Gutmann, F., and Hacobian, S. (1951).—*Aust. J. Sci. Res.* A **4**: 595.
Delahay, P. (1954).—"New Instrumental Methods in Electrochemistry." (Interscience Publishers Inc.: New York.)
Delahay, P., and Adams, T. J. (1952).—*J. Amer. Chem. Soc.* **74**: 5740.
Kalyanasundaram, A. (1951).—*Proc. Indian Acad. Sci.* A **33**: 316.
Loveland, J. W., and Elving, P. J. (1952).—*J. Phys. Chem.* **56**: 250.
Randles, J. E. B., and Somerton, K. W. (1952).—*Trans. Faraday Soc.* **48**: 937.

ALTERNATING CURRENT POLAROGRAPHY: TENSAMMETRIC INVESTIGATION OF ADSORPTION PROCESSES. UTILITY OF PHASE-ANGLE MEASUREMENTS

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Summary

Tensammetric measurements, based on interpretation of the non-faradaic alternating current flow under conditions of alternating current polarography, have been used to study the adsorption of various substances on mercury electrodes. Such measurements can be greatly improved by use of an apparatus arrangement which permits measurement of both the alternating current and the phase angle; from the latter, the series resistance and the capacity of the electrical double layer can be calculated. The application to inorganic systems involving retrograde currents and film formation is described.

I. Introduction

The term *tensammetry* has been used (Breyer and Hacobian 1952) to describe the study of adsorption processes through alternating current measurements, using the same type of experimental arrangement as in alternating current (A.C.) polarography; the measured alternating current flowing through the cell has been interpreted on the basis that it is proportional to the capacity of the electrical double layer (except in narrow ranges of potential where large pseudo-capacitative effects are present). A decrease in the current, therefore, other things being equal, implies a decrease in the capacity of the electrical double layer, and, hence, displacement of some of the hydrated ions in the layer by some other substance, that is, the presence of an adsorption process.

In tensammetric measurements, as in A.C. polarography, measurement of the alternating current alone does not permit precise calculation of the series resistance and the double layer capacity. However, use of an experimental arrangement by which both the alternating current and the phase angle can be measured enables the series resistance and the double layer capacity to be calculated precisely.

The apparatus previously described (Bauer and Elving 1959) has been used to evaluate the possible connection of adsorption processes with certain types of anomalous waves observed in conventional direct current polarography.

II. Basis for the Measurements

For an adsorption process where the rate of attainment of equilibrium is rapid, the solution-electrode system may be represented as shown in Figure 1.

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From the measured parameters, I_r, V, and θ (the phase angle between the alternating current through the cell and the alternating potential), the series resistance R_X and the double layer capacity C_H can be calculated by the following equations for a series combination of R_X and C_H:

$$\cot \theta = \omega C_H R_X, \qquad (1)$$

$$\frac{V}{I_r} = R_X^2 + \left(\frac{1}{\omega C_H}\right)^2, \qquad (2)$$

where ω is the angular frequency of the applied alternating potential.

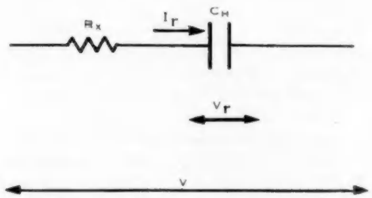

Fig. 1.—Equivalent circuit under conditions of alternating current polarography for an adsorption process where equilibrium is rapidly attained. V is the alternating potential across the cell, V_r the alternating potential across the interface, I_r the alternating current, R_X the series resistances (electrode plus electrolyte resistance), and C_H the capacity of the double layer.

In a case where diffusion processes play no rate-determining role, for example, a solution of an electrolyte such as KCl or $NaClO_4$ at a potential where oxidation or reduction of the electrolyte ions does not occur, the resistance R_X thus calculated depends only on the solution, electrode, and contact resistances, and C_H is the calculated capacity of the double layer. Comparison of C_H values will then show whether or not addition of some substances produces adsorption phenomena.

III. Adsorption in Retrograde Currents

Retrograde polarographic currents (e.g. Kivalo and Laitinen 1955) are generally distinguished by a sudden current drop as the potential is increased after an apparently normal limiting current has been attained. They have been observed under a variety of circumstances and a number of explanations have been advanced to account for them.

Some results on retrograde polarographic currents obtained in the present investigation are shown in Figures 2 and 3. In the presence of vanadate ion (Fig. 2 (*a*)), the double layer capacity is lowered on both sides of the first polarographic reduction wave (Fig. 2 (*b*)), showing that both oxidized and reduced forms are adsorbed. The small capacity peak (at *c.* $-0 \cdot 45$ V), corresponding to the polarographic wave, may be the outcome of a somewhat reversible faradaic process, or may be a non-faradaic phenomenon due to a change in constitution of the double layer over this potential range, brought about by replacement of the oxidized form by the reduced form as a result of the reduction process.

The plot of resistance, R_X, as a function of potential (Fig. 2 (*c*)) shows that the processes occurring also simulate a resistance; if R_X were only a series resistance, it would be independent of polarizing potential. It is apparent that, in the supporting electrolyte alone, there is also a process, which simulates the effect of a resistance, perhaps due to an interaction between the buffer component and the mercury surface. In addition, in the presence of vanadate, there is a resistance peak at $-0 \cdot 45$ V corresponding to the reduction wave. This resistance peak, like the capacity peak, may be the outcome of either a faradaic or non-faradaic process.

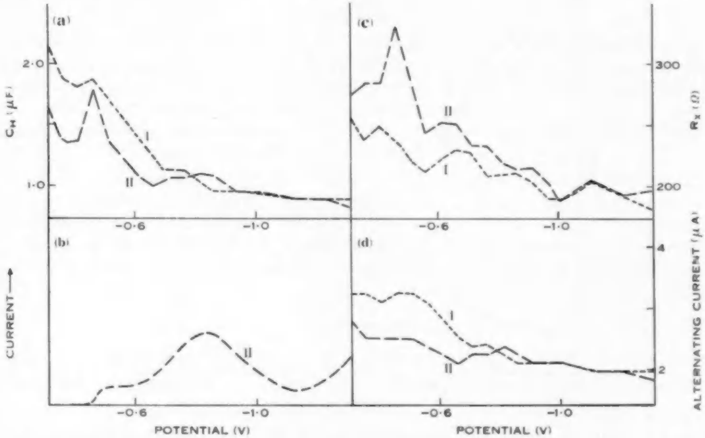

Fig. 2.—Vanadate ion (1mM) in phosphate buffer of pH 6·8 (potentials are given *v.* the saturated mercurous sulphate electrode, and are uncorrected for large *IR* drops (R ~10,000 Ω)). I, Background electrolyte only; II, vanadate solution.

(*a*) Double layer capacity as function of polarizing potential; (*b*) D.C. polarogram; (*c*) R_X as function of polarizing potential; (*d*) alternating current as function of polarizing potential. Curves (*a*) and (*c*) can only be obtained by measuring both current and phase angle; current measurement alone gives the less revealing curve (*d*).

It is noteworthy that the plot of alternating current against D.C. polarizing potential (Fig. 2 (*d*)) does not show a peak. This indicates the improvement in sensitivity resulting from measurement of both alternating current and phase angle, permitting calculation of C_H and R_X, as opposed to having only values for the alternating current flow through the cell.

IV. Film Formation

Figure 3 shows that the capacitive current due to the double layer has a marked hump (curve II), which is characteristic of chloride solutions. This hump disappears in the presence of arsenic (curve III), that is, the capacity of the double layer is decreased. It can be concluded that the reduction of arsenic leads to film formation at the electrode, an inference drawn previously on the basis of polarographic evidence (Meites 1954).

V. Criteria for Application and Interpretation

In studies of adsorption by tensammetry, it should be remembered that unless the calculated R_X values are independent of potential (as shown by the test earlier described), the circuit representation of Figure 1 is invalid and, consequently, C_H will not accurately represent the double layer capacity. With redox processes, a resistance and a capacity are simulated by the periodic diffusion

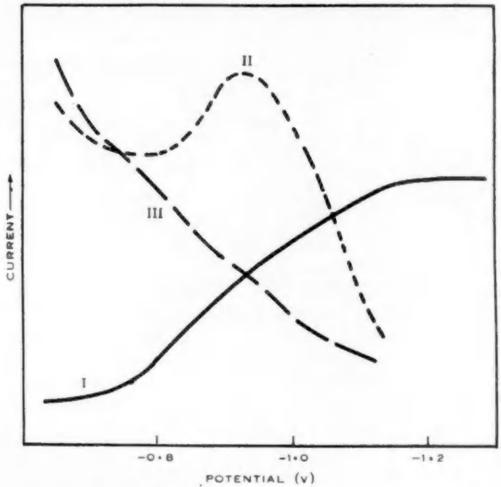

Fig. 3.—Solution of 1mM As(III) in 1M HCl (potentials are given *v.* the saturated mercurous sulphate electrode, and are uncorrected for large *IR* drops ($R \sim 10{,}000\ \Omega$)). I, D.C. polarogram; II, A.C. polarogram for base electrolyte alone; III, A.C. polarogram for solution containing arsenic.

phenomena resulting from the alternating potential. Similarly, one would expect to find cases, where the rate of attainment of adsorption equilibrium is to some extent diffusion controlled, and where, consequently, additional circuit elements must be included in Figure 1. Nevertheless, it seems safe to say that, when C_H, as calculated from equations (**1**) and (**2**), shows a lowering, this decrease in capacity is associated with some non-faradaic process affecting the interface.

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VII. References

Bauer, H. H., and Elving, P. J. (1959).—*J. Amer. Chem. Soc.* (in press).
Breyer, B., and Hacobian, S. (1952).—*Aust. J. Sci. Res.* A **5**: 500.
Kivalo, P., and Laitinen, H. A. (1955).—*J. Amer. Chem. Soc.* **77**: 5205.
Meites, L. (1954).—*J. Amer. Chem. Soc.* **76**: 5927.

REFINED ANTISYMMETRIC MOLECULAR–ORBITAL CALCULATIONS OF THE ENERGY LEVELS OF BENZENE AND HEXAMETHYLBENZENE

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Summary

The calculation by Parr, Craig, and Ross (1950) of the lower π-electron energy levels of benzene has been repeated, with specific inclusion this time of (i) the contribution of the hydrogen atoms to the core potential, and (ii) exchange interactions between the π-electrons and the core. As a result, the lower energy levels (in the range 4–10 eV) are all raised by amounts averaging about 1 eV. A similar result was obtained by Niira (1953), but by a significantly different procedure.

If the hydrogen atoms are replaced by methyl groups, the changed core potential reduces the energy level spacing slightly. This effect (including an allowance for the polarities of ring-radical σ-bonds) can account for about 35 per cent. of the difference in energy (estimated at 3800 cm^{-1}) between the $^1B_{2u}$—$^1A_{1g}$ transitions in benzene and hexamethylbenzene ; it is thus almost as important in this context as hyperconjugation and like effects.

I. Introduction

The non-empirical molecular-orbital calculation of the energy levels of benzene has been carried out with many variations, a convenient summary of which has been assembled by Kauzmann (1957). Yet despite their variety, all these calculations still incorporate many approximations, at whose importance we can only guess. We recount here the results of yet another version, in which certain improvements have been incorporated, not necessarily in the expectation that they will yield better agreement with experiment (such agreement as has already been achieved must be partly fortuitous), but rather with the intention of assessing quantitatively the importance of certain small, hitherto neglected terms in the total molecular Hamiltionian. These terms all refer to the interaction between the π-electrons and the rest of the molecule—the " core "—and should properly be included in the treatment of any conjugated π-electron system, so that the matters dealt with here are of some generality.

The effect of these terms we assess by comparison with two, only, of the earlier treatments. The first is that of Parr, Craig, and Ross (1950), henceforth abbreviated to PCR, who neglected the hydrogen atoms completely and who further omitted the exchange part of the potential due to the σ-electrons. The other is Niira's (1953) treatment, in which the exchange interactions of all the core electrons (including those of the hydrogens) were included, though by a procedure of which we have some criticisms. In the present case we have

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included fully all coulomb and exchange interactions between the π-electrons and the core (though we do not follow Niira in describing the inclusion of exchange terms as σ–π interaction: it seems to us that in the context of molecular-orbital theory this term should be reserved for the effect of interaction between low-energy states and configurations in which at least one σ-electron is excited (cf. Ross 1952)). We also go one step further. It is well known that the replacement of hydrogen atoms by alkyl groups significantly shifts the energy levels of conjugated systems; there is a qualitative, but no strictly non-empirical and quantitative theory of this effect, which is largely attributed to valence interaction between the ring and the α-hydrogen atoms of the side-chain, for example, via hyperconjugation (Mulliken, Rieke, and Brown 1941) or α-hydrogen bonding (Kreevoy and Eyring 1957). A part, however, of this effect must be due simply to the slight change in the core potential, and this we can assess by replacing the hydrogen atoms in our earlier calculation by methyl groups.

II. Orbital Energies

The essential details of these calculations are given in Appendix I; the results are summarized in Tables 1 and 2. The latter table refers to actual molecular energy levels; the former, more simply, to the orbital energies of individual electrons. There are six independent π-electron molecular orbitals, denoted in order of increasing energy by φ_0, $\varphi_{\pm 1}$ (degenerate), $\varphi_{\pm 2}$ (degenerate), φ_3. By the orbital energy ε_j is meant the kinetic energy of an electron in orbital φ_j, plus its potential energy in the field of the core. The energy of a particular

Table 1

orbital energies (ev)

	PCR	(*a*)	(*b*)	(*c*)	(*d*)
Core	C_6	C_6	C_6	C_6H_6	C_6Me_6
	No Exchange Included		Exchange Included		
ε_3—ε_2 ..	2·754	4·792	3·670	4·405	4·348
ε_2—ε_1 ..	5·358	5·444	6·582	6·242	6·160
ε_1—ε_0 ..	2·932	1·688	3·010	3·286	3·220
ε_3—ε_0 ..	11·044	11·924	13·262	13·933	13·728

π-electron configuration is the sum of six such orbital energies, plus the mutual interaction energies of the six π-electrons; inclusion of configuration interaction (CI) then gives the final molecular energy levels. Since we are here concerned only with different ways of calculating the orbital energies ε_j, it is largely sufficient to tabulate them only.

The first column of Table 1 quotes from PCR. Column (*a*) gives the results of a closely similar calculation, but with two procedural changes, which were introduced to simplify the work, but which are also probably physically realistic as well. They are: the use of Mulliken's (1949) instead of London's (1945)

approximation for 3-centre integrals; and the use of nodeless, instead of hydrogen-like, carbon $2s$ atomic orbital wavefunctions. Column (*a*) thus represents PCR's results, recalculated in a way that makes them suitable for comparison with the results of the subsequent calculations. It was not, it is true, expected that this seemingly trivial revision should disturb the spacing of

TABLE 2

RELATIVE ENERGY LEVELS OF BENZENE (ev)

Level and Dominant Configuration	PCR†		(*a*)†		(*c*)†		Experimental	
	No CI	CI	No CI	CI	No CI	CI	(%)	Assign-ments
Singlets								
A_{1g} ..	0	0 (—2·7)‡	0	0 (—2·6)	0	0 (—2·4)	(13)§	0·0
$A_{2g}(1\rightarrow2)^{2*}$ ..	11·1	12·5	11·2	12·6	12·8	14·0		—
$B_{1u}(1\rightarrow2)^{*}$ ..	7·3	9·0	7·3	9·1	8·1	9·7		(6·2)‖
$B_{2u}(1\rightarrow2)$ ..	5·9	4·4	6·0	4·5	6·8	5·3	(19)	4·9
$E_{1u}(1\rightarrow2)^{*}$ ..	9·8	9·9	9·9	9·9	10·7	10·6	(12)	7·0
$E_{2g}\begin{cases}(1\rightarrow2) \text{ or}\\(0\rightarrow2)\end{cases}$	10·9	7·6	9·7	7·5	12·1	9·2	(12)	(6·2)‖
Triplets								
$A_{1g}(1\rightarrow2)^{2*}$ ..	13·9	15·2	14·1	15·5	15·7	17·0		
$A_{2g}(1\rightarrow2)^{2}$ ..	12·6	11·5	12·8	11·4	14·4	13·2		—
$B_{1u}(1\rightarrow2)$ ..	3·1	3·6	3·2	3·7	4·0	4·3	(9)	3·8
$E_{2u}(1\rightarrow2)^{*}$ ..	5·8	8·2	5·8	8·3	6·6	8·8		—
$E_{1u}(1\rightarrow2)$ ..	4·4	4·5	4·5	4·5	5·3	5·4	(17)	—
$E_{2g}\begin{cases}(1\rightarrow2) \text{ or}\\(0\rightarrow2)\end{cases}$	8·3	6·3	7·1	6·3	9·5	7·8	—	

* Denotes state incompatible with non-polar valence bond structures.
† Notation of Table 1.
‡ Figure in parentheses is the depression of the ground-state energy due to CI.
§ Percentage of higher energy configurations in complete state wavefunctions.
‖ Alternative assignments.

the one-electron levels so much, and in a manner which, since it upsets the expected approximate equality between $\varepsilon_3-\varepsilon_2$ and $\varepsilon_1-\varepsilon_0$, seems physically unlikely. Numerically, the changes are practically entirely due to the new values of a very few 3-centre integrals for which our *a priori* expectation was that the Mulliken approximation should be particularly suitable.* That such small changes, none exceeding 0·3 eV, can have such far-reaching consequences is yet another reminder that the energy quantities here recorded emerge as small differences

* These are integrals, of which the penetration integral (1 : 26)—see Appendix I—is typical, in which one electron is distributed between two centres whose mid-point is close to the interacting atom, nucleus, or electron. Such integrals are included in Barker and Eyring's (1954) evaluation of multicentre integral approximations, for the case of integrals over $1s$ atomic orbitals which they were able to compute exactly.

between large numbers, and that their absolute values, particularly, must be interpreted with due caution.

Column (*b*) of Table 1 shows the effect of including the exchange, as well as the coulomb, interactions of the core carbon electrons. Besides, happily, restoring the symmetry of the energy level pattern, the principal effect is to spread the levels somewhat further apart (cf. the last line of Table 1); this occurs because the exchange terms, whose signs in this situation are all opposite to those of the electronic coulomb terms, effectively constitute an additional attractive contribution to the core potential. So, too, do the hydrogen atoms, when they are included, and thus, in column (*c*), the energy levels are even further separated. In (*d*), which refers to a simplified model of hexamethylbenzene, there have been inserted, in place of the hydrogens, six carbon atoms (at the proper bond distance of 1·54 Å), constructed from a nucleus of charge +4 and four electrons in the atomic configuration $2s2p_x2p_y2p_z$. The hydrogens of the methyl groups are too remote to contribute significantly.

In each of these last two calculations the bonds from ring to radicals were assumed to be strictly covalent. Recently proposed values (Petro 1958) for the electric dipole moments of the relevant bonds (negative end written first) are 0·63 D for $C(sp^2)-H$ and 0·69 D for $C(sp^2)-C(sp^3)$. These figures differ markedly, even in sign, from the calculated moments derived from simple covalent two-electron σ-bond wavefunctions (−0·8 and −0·04 D, respectively). Thus, despite the uncertainty regarding the true meaning of the experimental bond moments, and the proper way of representing the actual charge dissymmetries, it would seem reasonable to contemplate adding say 10 per cent. ionic character (C^-H^+ or $C^-CH_3^+$)—dipole moment about 0·6 D—to the bond wavefunctions. The consequences of doing this were explored, and found to be small, especially for the molecular orbitals of lower energy. Specifically, the entries in column (*c*)—C_6H_6—of Table 1 were decreased, in order downwards, by 0·238, 0·008, 0·002 (and 0·248) eV and those in column (*d*)—C_6Me_6—by 0·238, 0·064, 0·026 (and 0·328) eV. These figures are offered as reasonable upper estimates of the effect of σ-bond polarity, and for future reference attention is particularly drawn to the fact that in comparing C_6Me_6 with C_6H_6, the change in $\varepsilon_2-\varepsilon_1$ is highly insensitive to whatever assumptions we make about the CH bond polarity.

III. Benzene Molecular Energy Levels

In Table 2 we give revised values of the lower molecular energy levels, with and without CI. While only the lowest state of each species is given, we checked that there are no other important low-energy states, the gap (after CI) between the two lowest states of each type being between 5 and 10 eV. The first column of the table lists these types, using the usual notation for the irreducible representations of poing group D_{6h}, and in addition the lowest-energy configuration is specified by citing the promotion required from the ground state 0^21^4 ($\varphi_0^2\,\varphi_{\pm1}^4$). There then follow PCR's results, with some numerical corrections. The figures in the columns corresponding to calculation (*a*) of Table 1 are very similar to the PCR values because, rather happily, $\varepsilon_2-\varepsilon_1$ was scarcely altered by the procedural difference between the two calculations; the lower excited

levels are largely sensitive to this orbital energy difference alone. The more complete calculation (c) reveals larger differences. The excited states are all raised by about 1 eV, and on the whole agreement with experiment is slightly impaired, since all calculated levels which can be compared with experiment are now too high. However, the pattern could easily be compressed somewhat by slightly reducing the effective nuclear charge of carbon for the $2p_\pi$ electrons—the value used, $Z=3\cdot18$ is Zener's (1931) early estimate and may well be too large, especially for the excited states.

Rather more interestingly, the new calculation brings the energies of the singlet B_{1u} and E_{1u} states much closer together, thus attenuating the support which this calculation has given to the assignment ${}^1E_{2g}-{}^1A_{1g}$ for the 2000 Å transition in benzene (cf. Dunn and Ingold 1955).* Craig (1951) has already pointed out that certain states (appropriately marked in the table) cannot be generated, in valence bond theory, from purely covalent wavefunctions. Our procedure is known to overestimate the energies of these ionic states, and since the calculated energy of ${}^1E_{1u}$ is too high, so also presumably is that of ${}^1B_{1u}$. This is confirmed by Hurley's (1958) calculation, which employed a refined procedure, designed to avoid these overestimates of ionic-structure energies. In Hurley's calculation, as also in the markedly successful semi-empirical theory of Pariser (1956), ${}^1B_{1u}$ is definitely the lower state. However, we further point out that the effect of CI on the E_{2g} state is particularly drastic, and sensitive to the approximations used, since before CI there are several states of almost equal low energies. Figures, therefore, for E_{2g} energies should not be taken too literally, but it now appears that the weight of theoretical evidence favours ${}^1B_{1u}$ as the lower state, and thus as the upper state of the 2000 Å benzene absorption system.

Finally, as a measure of the importance of CI in this calculation, we include, for the last calculation, and the most important states, the summed weights of configuration wavefunctions, other than the lowest, in the final state wavefunctions.

We must now comment on the results obtained by Niira (1953), who, as mentioned earlier, also carried out a calculation of this kind, on benzene, in which exchange was included. He found, as do we, an expansion in the energy level pattern, but as he did not specifically cite ε_j values, we have not included any of his figures in our table. However, because his method of including configuration interaction was particularly rough, it is possible to work backwards from his table of molecular energy levels, to the result that his figure for $\varepsilon_2-\varepsilon_1$ is $6\cdot4$ eV, which is close to our figure in column (b) of Table 1. This, however, is largely coincidental, since his procedure and ours differed significantly. On the one hand, Niira omitted nearly all the 3-centre exchange integrals, and those he did include (the second of the two types which receive special mention in Appendix I) were approximated to by Ruedenberg's (1951) second method, which Barker and Eyring (1954) find unsatisfactory. Also, he omitted the

* It has twice been claimed (Moffitt and Scanlan 1953; Hurley 1958) that there was an error in the original (PCR) calculation of the ${}^1E_{1u}$ energy, after CI. We have, however, exhaustively checked this calculation and are confident that the original figure was correct.

coulomb part of the hydrogen atom interactions, whose net effect is, in fact, just as important as the exchange contribution and has the same sign. On the other hand, he did specifically include, as we have not, the exchange energy of a π-electron, localized on a particular nucleus, with the other electrons on the same nucleus, and this is a sizeable quantity. It is our belief, as stated in the formal derivation of the expression for the orbital energies which we have used in this work (Ross 1952), that this is properly regarded as part of a constant energy term, the energy of a $2p_\pi$ electron in an isolated carbon atom, which is common to all the ε_j, and disappears in differences between them. Niira's procedure is thus equivalent to assuming that his $2p_\pi$ orbitals are eigenfunctions of a Hartree potential, and then evaluating the energy with Fock's exchange terms added; ours to the assumption that these atomic orbitals satisfy the Hartree-Fock equation at the outset. The terms Niira included, and those with which we have been concerned, though largely different, are all in the nature of an additional attraction between the core and the π-electrons, which is why our final results are rather similar.

IV. Hexamethylbenzene

The calculation on hexamethylbenzene admits of one comparison with experiment. This is the shift, relative to benzene, of the ${}^1B_{2u}-{}^1A_{1g}$ (2600 Å) transition. This shift should be given approximately by the difference between the values $\varepsilon_2-\varepsilon_1$ in the calculations (*c*) and (*d*) of Table 1 (0·082 eV)—assuming, as we have done throughout, no direct interaction of the hyperconjugative, or similar, type. Taking configuration interaction into account, the predicted shift becomes 0·089 eV, or 720 cm^{-1}, towards lower frequencies.

Experimentally, we estimate the shift of the origin of the 2600 Å band as about 3000 cm^{-1}. This figure has been arrived at indirectly, since the vapour spectrum in this region (of which there is no report in the literature: we are indebted to Dr. G. R. Hunt for taking some photographs of it for us) is virtually devoid of vibrational structure. This is doubtless due to internal rotation of, and steric hindrance between, the six methyl groups. The possibility of such interferences was completely neglected in our simple model of the molecule. Our estimate is therefore derived by extrapolating, to six methyl substituents, the data for substituted benzenes having fewer methyl groups in non-adjacent positions; namely, toluene (red shift of 610 cm^{-1}; Ginsburg, Robertson, and Matsen 1946), the mean of *m*- and *p*-xylene (1937, 1135 cm^{-1} respectively, Cooper and Sponer 1952; Cooper and Sastri 1952), and mesitylene (1533 cm^{-1}, Sponer and Stallcup 1948).

Nevertheless, this figure is still not really the quantity to which the calculations refer, which is the vertical excitation energy from the vibrationless ground state. To relate these two excitation energies, in the general case in which the substitution changes the equilibrium geometries and the shapes of the vibrational potential energy surfaces, is quite an elaborate problem, and one which, to our knowledge, has never been contemplated in treatments of substitutional effects in spectra. Here, however, we can reasonably assume that there is no important change of this kind, and consider only the effects of zero-point energies. In

benzene, in the ground state, the zero-point energy, according to Garforth, Ingold, and Poole (1948) is 21535 cm^{-1}; in the upper state it is reduced by 1485 cm^{-1}. In benzene-d_6, all the frequencies are lowered and the reduction in zero-point energy is thereby itself lowered by 208 cm^{-1}. That is, complete deuteration results in a shift of the origin, towards the blue, of 208 cm^{-1}. Complete isotopic substitution by hypothetical nuclei of infinite mass would eliminate the zero-point vibration effect entirely, and thus result in a blue shift of 1485 cm^{-1} which sets an upper limit to the zero-point energy contribution to the substitutional shift of the band origin. Assuming that the methyl groups may be treated as point masses of atomic weight 15—that is, that the CH_3 frequencies do not significantly change on excitation—we estimate by a rough calculation that cannot be far amiss that the zero-point energy of hexamethylbenzene drops by about 800 cm^{-1} on excitation to the $^1B_{2u}$ state; that is, that hexamethyl substitution should, due to this effect alone, produce a blue shift of about 800 cm^{-1}.

The observed red shift of the origin therefore corresponds to a red shift of about 3800 cm^{-1} in the vertical excitation energies. Comparison with our calculated figure shows that about 20 per cent. of this can be attributed to the change in the core potential, assuming that the ring-substituent σ-bonds remain perfectly homopolar. In the light of our earlier considerations on the effect of polarity on the orbital energy difference $\varepsilon_2 - \varepsilon_1$, we suggest that any polarity contribution is upwards of 0·06 eV or 15 per cent. of the total shift of origin, and also towards the red. It is therefore clear that the effects we have dealt with here can account for about 35 per cent. of the total red shift in hexamethylbenzene; direct interaction, in the sense of mutual delocalization, between the ring and the electrons in the CH bonds of the methyl groups, must be responsible for the rest. That is, the revised figure, including a rough estimate of its error, for the red shift due to the delocalization processes, becomes 2500 ± 250 cm^{-1}. On the other hand we emphasize that the effects we are dealing with here are of about the same order of importance as the widely recognized delocalization processes.

V. References

Barker, R. S., and Eyring, H. (1954).—*J. Chem. Phys.* **22**: 2072.

Cooper, C. D., and Sastri, M. L. N. (1952).—*J. Chem. Phys.* **20**: 607.

Cooper, C. D., and Sponer, H. (1952).—*J. Chem. Phys.* **20**: 1948.

Craig, D. P. (1951).—*Disc. Faraday Soc.* **9**: 5.

Dunn, T. M., and Ingold, C. K. (1955).—*Nature* **176**: 65.

Garforth, F. M., Ingold, C. K., and Poole, H. G. (1948).—*J. Chem. Soc.* **1948**: 508.

Ginsburg, N., Robertson, W. W., and Matsen, F. A. (1946).—*J. Chem. Phys.* **14**: 511.

Hurley, A. C. (1958).—*J. Chem. Phys.* **28**: 532.

Kauzmann, W. (1957).—" Quantum Chemistry." p. 472. (Academic Press Inc.: New York.)

Kopineck, H. J. (1952).—*Z. Naturf.* **7a**: 785.

Kotani, M., Amemiya, A., Ishiguro, E., and Kimura, T. (1955).—" Table of Molecular Integrals." (Maruzen Co. Ltd.: Tokyo.)

Kreevoy, M. M., and Eyring, H. (1957).—*J. Amer. Chem. Soc.* **57**: 5121.

London, A. (1945).—*J. Chem. Phys.* **13**: 396.

Moffitt, W., and Scanlan, J. (1953).—*Proc. Roy. Soc.* A **220**: 530.

Mulligan, J. F. (1951).—*J. Chem. Phys.* **19**: 347.

Mulliken, R. S. (1949).—*J. Chim. Phys.* **46**: 497.

Mulliken, R. S., Rieke, C. A., and Brown, W. G. (1941).—*J. Amer. Chem. Soc.* **63**: 41.
Niira, K. (1953).—*J. Phys. Soc. Japan* **8**: 630.
Pariser, R. (1956).—*J. Chem. Phys.* **24**: 250.
Parr, R. G., Craig, D. P., and Ross, I. G. (1950).—*J. Chem. Phys.* **18**: 1561.
Petro, A. J. (1958).—*J. Amer. Chem. Soc.* **80**: 4230.
Ross, I. G. (1952).—*Trans. Faraday Soc.* **48**: 973.
Ruedenberg, K. (1951).—*J. Chem. Phys.* **19**: 1433.
Sklar, A. L. (1939).—*J. Chem. Phys.* **7**: 984.
Sponer, H., and Stallcup, M. J. (1948).—" Vol. Comm**é**m. Victor Henri 1947/48." pp. 211–22. (Desoer: Liége.)
Zener, C. (1931).—*Phys. Rev.* **36**: 51.

Appendix I

We need only describe those details of the calculation which have not already been given by PCR. The greater part of the present work lay in the evaluation of over two hundred 2- and 3-centre one- and two-electron integrals over Slater atomic orbital wavefunctions. The 2-centre integrals were mostly obtained exactly, using the formulae and tables of Kopineck (1952 and previous papers) and of Kotani *et al.* (1955) (PCR's figures were thereby slightly revised), the exceptions being the heteronuclear carbon-hydrogen integrals. For these, bivariate interpolation in Kotani's tables was used, or else, for the very small ones, an approximation due to Sklar (1939). Three-centre integrals were approximated to by Mulliken's method, concerning which we make two comments, since we believe we have used his formula in cases rather more complicated than those to which it has hitherto been applied:

(i) When the three centres are not collinear, and one at least of the atomic orbitals is a $2p$ in-plane (σ) orbital, then its axis of quantization is in general not appropriate to some, or even any, of the 2-centre overlap and coulomb integrals which Mulliken's formula employs. The formula must therefore be considerably elaborated by resolving such $2p_\sigma$ orbitals into properly oriented components.

(ii) Mulliken's method breaks down when the overlap integrals vanish by symmetry. There are several possible ways out of this difficulty. An alternative formula, due to Ruedenberg (1951), has the advantage of not vanishing identically in such cases, but its performance, when tested on the simplest three-centre cases (Barker and Eyring 1954) or on known 2-centre integrals, is not encouraging. The same held when, following a suggestion of Mulligan (1951), the vanishing overlap integrals were replaced by transition moment integrals (whereupon various other devices are needed to restore the correct dimensions); this procedure, which we explored at some length, we deem unpromising. Our eventual method was somewhat empirical: in these refractory cases the vanishing overlap integrals were replaced by factors which give the correct results when the 3-centre integrals degenerate to 2-centre exchange integrals. For example, in Ruedenberg's notation, we set

$$(\chi_{a\pi}\chi_{cs} \mid \chi_{b\pi}\chi_{cs}) = \tfrac{1}{4}S'(a_\pi c_s)S'(b_\pi c_s)\{(\chi_{a\pi}\chi_{a\pi} \mid \chi_{b\pi}\chi_{b\pi}) + (\chi_{a\pi}\chi_{a\pi} \mid \chi_{cs}\chi_{cs}) + (\chi_{cs}\chi_{cs} \mid \chi_{b\pi}\chi_{b\pi}) + (\chi_{cs}\chi_{cs} \mid \chi_{cs}\chi_{cs})\},$$

where

$$\{S'(a_\pi c_s)\}^2 = 4(\chi_{a\pi}\chi_{cs} \mid \chi_{a\pi}\chi_{cs})\{(\chi_{a\pi}\chi_{a\pi} \mid \chi_{a\pi}\chi_{a\pi}) + (\chi_{cs}\chi_{cs} \mid \chi_{cs}\chi_{cs}) + 2(\chi_{a\pi}\chi_{a\pi} \mid \chi_{cs}\chi_{cs})\}^{-1}.$$

We are hopeful that this approximation, which is at least simple, is also realistic.

To include exchange in the core potential we used the formula for orbital energies already given by one of us (Ross 1952, eqn. (**15′**)); the same paper also prescribes the procedure for the case when the core bonds are heteropolar (the carbon atomic σ-bond orbitals in this case were taken as the appropriate *sp* hybrids).

From all these intermediate results a few are of sufficiently general applicability to be worth quoting. These, given in Table 3, are values of the so-called penetration integrals, themselves sums of the simpler integrals referred to above.

Table 3

PENETRATION INTEGRALS (eV) INCLUDING EXCHANGE*

Integral	Ring Carbon	Substituent Hydrogen	Substituent Carbon	Integral	Ring Carbon	Substituent Hydrogen	Substituent Carbon
1 : 11	—	0·995	0·545	1 : 23	0·220	0·001	0·003
1 : 22	1·669	0·020	0·027	1 : 24	0·074	0·000	0·001
1 : 33	0·035	0·000	0·001	1 : 25	0·119	0·001	0·001
1 : 44	0·007	0·000	0·000	1 : 34	0·011	0·000	0·000
				1 : 35	0·019	0·000	0·000
1 : 12	3·943	0·099	0·084				
1 : 13	0·346	0·012	0·011				
1 : 14	0·136	0·005	0·005				

* Slater atomic orbitals, with effective nuclear charges $Z=3\cdot18$ (for carbon $2s$, $2p$) and $Z=1\cdot00$ (for hydrogen $1s$).

The penetration integral $(i:jk)$ is the attractive energy between a charge distribution $j_\pi k_\pi$ (j_π and k_π denote Slater $2p_\pi$ atomic orbitals of the jth and kth carbon atoms) in the coulomb-plus-exchange field of the ith ring, or substituent, atom (if a carbon atom, the configuration is $2s2p_x2p_y2p_z$). The carbon atoms are numbered in order around the ring, as are the substituents. The values in the " ring carbon " atom column of Table 3 are about twice as large as those given by PCR, because of the inclusion of exchange.

Apart from the calculation of the orbital energies, the evaluation of the complete molecular state energies followed PCR precisely, except that all the secular equations were solved exactly using the electronic digital computer SILLIAC.

COMPLEX FORMATION BY INORGANIC IONS

By J. F. Duncan*

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Summary

The formation of complexes $MN^{(m-n)+}$ from M^{m+} and N^{n-} in aqueous solution is discussed in terms of simple electrostatic forces. The entropy changes are shown to depend on similar factors to those which determine the enthalpy changes. In cases where the entropy changes for a series of complexes with different M^{m+} and constant N^{n-} are either linearly related to the enthalpy changes, or can be neglected, the free energy of formation of the complex from the ions may be predicted in unknown cases. The relations commonly used for plotting the free energy of formation of the complex from the ions as functions of a number of physicochemical quantities are shown to arise fortuitously as a consequence of the restricted range of ionic radii provided by Nature. In some cases deviations from expected behaviour show the influence of structural changes on the ease of complex formation.

I. Introduction

Although numerous stability constants of metal complexes are known, little attempt has been made to understand the variations of stability constant which occur with change of metal ion or ligand. Irving and Rossotti (1956) have given several examples of the types of variation which can be obtained ; other workers have related the thermodynamic properties of metal complexes to the ionization potential of the metal (Freiser 1952), and to functions of the ionic radius (Nancollas 1956). In this contribution, we discuss how far such relations are justified, and point out the significance of anomalies. We have used an electrostatic model to evaluate the various energy terms. This procedure is justified by its simplicity, and its marked success in interpreting absorption spectra and molecular structure (Ballhausen 1954, 1955; Jorgensen 1954, 1955*a* 1955*b*). In using it, however, we do not wish to imply that the bonds between the metal ion and the ligand are wholly ionic, although they have been treated as such. The alternative, and more realistic treatment, based on bond orbital approximations, is not so easily applied to a large number of different ions and ligands, the wavefunctions of which are often not known with any certainty.

II. Theory

Consider the reaction

$$M^{m+}(aq)+N^{n-}(aq)=MN^{(m-n)+}(aq), \qquad \ldots\ldots\ldots\ldots \quad (1)$$

where M^{m+} is the metal ion, and N^{n-} is the singly coordinated ligand. We are concerned with the evaluation of the free-energy change ΔG^0 of this reaction

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in terms of parameters characteristic of M and N. The enthalpy and entropy changes will be considered separately in a manner similar to that used by Eley and Evans (1938) in evaluating the heats of hydration of metal ions.

(a) *Enthalpies*

The enthalpy change in reaction (1) can be evaluated by performing a thermodynamic cycle as follows:

(i) The two ions M^{m+} and N^{n-}, complete with their coordinated water molecules (p on the cation, and q on the anion), are removed from the solution into the gas phase. An expenditure of energy (A) on the system is required.

(ii) The coordinated water molecules are removed from each of the ions. The energy per water molecule necessary to overcome the electrostatic attraction to the cation is

$$\Delta H_{M^{m+}} = \Sigma m\alpha e^2/rD, \qquad (2)$$

where r is the distance between the centres of the ion and either the hydrogen atoms, or the oxygen atoms, of the water molecule, on which reside fractional charges α. D is the dielectric constant. A similar equation obtains for $\Delta H_{N^{n-}}$. To evaluate these terms we need r. For cation hydration, we have, for the oxygen atom,

$$r = r_i + r_w,$$

where r_i and r_w are respectively the radii of the ion, and the water molecule; and for the hydrogen atom,

$$r^2 = 2r_w(r_i + r_w)(1 + \cos\theta/2) + r^2_{M^{m+}}, \qquad (3)$$

where θ is the angle between the two O—H bonds of the water molecule. For anion hydration, one of the O—H bonds is along the line of centres of the oxygen atom, and the ion N^{n-}. Hence, for one H atom,

$$r = r_{N^{n-}} + r_w,$$

and for the other

$$r^2 = 2r_w(r_{N^{n-}} + r_w)(1 + \cos\theta) + r^2_{N^{n-}}.$$

(iii) The two unhydrated ions are now brought into adjacent positions, so that each becomes part of the coordination sphere of the other. The energy liberated is now

$$E = mne^2/(r_{M^{m+}} + r_{N^{n-}})D. \qquad (4)$$

(iv) The $p+q$ water molecules are now arranged round the species $MN^{(m-n)+}$ in their normal coordination positions. We do not specify how this shall be done. But there will often no longer be room for all the water molecules to be adjacent to one of the two ions. A number, s, will have to be part of a second coordination sphere. They can be regarded as free water molecules, since the electrostatic potential energy of them will be much smaller than those adjacent to either of the ions. We are left, then, with a liberation of energy equal to the coordination energy of the $p+q-s$ water molecules, a fraction f of which will be coordinated to the cation. It is also possible that the structure may be so distorted that a

single water molecule may fill a coordination position on both the cation and the anion simultaneously (Duncan and Kepert 1959). This would affect the water reorientation energy (*vide infra*).

(v) The species $MN^{(m-n)+}$ is now returned to the aqueous solution, with an energy change B. Neither A ((i) above) nor B can be directly evaluated, but the difference between them is determined by the following:

(1) The difference in energy of Born charging, E_B (Born 1920) for the species $MN^{(m-n)+}$ and the separated ions: These are respectively the differences in electrostatic energy *in vacuo*, and in the aqueous solution of dielectric constant D of the dipole $MN^{(m-n)+}$ and the separated ions M^{m+} and N^{n-}. Hence,

$$E_B=\frac{1}{2}\left\{\frac{m^2e^2}{r_{M^{m+}}+2r_w}+\frac{n^2e^2}{r_{N^{n-}}+2r_w}-\frac{mne^2}{r_{M^{m+}}+r_{N^{n-}}+2r_w}\right\}\left\{1-\frac{1}{D}\right\}. \quad .. \quad (5)$$

In this equation it is assumed that the sphere to be charged is, in the case of the separate ions, equal to the sum of the unhydrated radius of the ions, and the diameter of a water molecule—that is, that the charge is on the surface of a sphere enveloping the oxygen atoms. This procedure, followed by Eley and Evans (1938), has led to reasonable agreement between the calculated hydration energies of ions and the experimental values. In the Eley-Evans' estimates, the energy of Born charging is usually smaller than the electrostatic coordination energy. On the other hand, Latimer (1926, 1935), Latimer and Buffington (1926), Laidler (1956), and Laidler and Couture (1956) use unhydrated radii to evaluate the entropies of aqueous ions. The last authors obtained quite reasonable agreement with the predictions of the Born equation. Use of unhydrated radii has the great advantage that it is simple, although it clearly cannot represent the *status quo* very precisely, since water coordination is ignored. Since we are concerned here to identify the parameters which determine ΔH^0, the enthalpy change in reaction (**1**), we have followed Eley and Evans in estimating the Born charging energy. In treating the dipole $MN^{(m-n)+}$, a separation of charge at a distance $r_{M^{m+}}+r_{N^{n-}}+2r_w$ has been assumed.

(2) The reorientation energy of the water molecules: The arrangement and orientation of the water molecules about the species $MN^{(m-n)+}$ may well be different from that about the individual ions. This quantity E_R may be estimated from models (Duncan and Kepert 1959) to be of the order of 18 kcal/mole water molecules undergoing reorientation.

(vi) The remaining s water molecules are returned to the solution with an energy liberation of sL, where L is the latent heat of vaporization of water.

We have now formed the species $MN^{(m-n)+}$(aq) by a process in which all the energy terms are known. The total energy change for the first stage of an association reaction between the ions M^{m+} and N^{n-} (i.e. for the formation of $MN^{(m-n)+}$) may be written

$$\begin{aligned}\Delta H^0 &= p\Delta H_{M^{m+}}+q\Delta H_{N^{n-}}-E-(p+q-s)f\Delta H_{M^{m+}}-(1-f)(p+q-s)\Delta H_{N^{n-}}\\&\quad +E_B+E_R-sL\\&=[p(1-f)-(q-s)f]\,\Delta H_{M^{m+}}+[fq-(1-f)(p-s)]\Delta H_{N^{n-}}\\&\quad -E+E_B+E_R-sL. \quad \ldots\ldots\ldots \quad (6)\end{aligned}$$

In this equation, we have combined an enthalpy term (sL) with a number of electrostatic terms. The latter are strictly free-energy terms, since they are evaluated from the work done in bringing together charges originally separated at infinity. They become equal to the enthalpy changes only when the temperature is 0 °A. The thermodynamic cycle above therefore refers to an operation performed at 0 °A. with an aqueous solution having the properties (dielectric constant etc.) it would have at any standard temperature we choose. However, the errors are small, when the relations are applied to aqueous solutions at room temperature.

Another feature which must be considered is the polarizability of the aquo-ions. In the above treatment, the possibility of formation of an induced dipole has been neglected. This is justified by the results of Eley and Evans, but other authors have found it necessary to include both the polarizability and the mutual repulsion energy of the dipoles of the water molecules. Thus, the enthalpies of formation of a number of inorganic compounds may be calculated on the electrostatic model using equations such as the following:

$$\Delta H^0 = \frac{-6Z(\mu_0+\mu_i)}{(r_{M^{m+}}+r_w)^2} + \frac{6(1\cdot19)(\mu_0+\mu_i)^2}{(r_{M^{m+}}+r_w)^3} + \frac{6\mu^2}{2\pi} + \frac{6B}{(r_{M^{m+}}+r_w)^9}, \quad \ldots \quad (7)$$

where μ_0 and μ_i are respectively the permanent and the induced dipole; π is the polarizability, and B is a constant (see Basolo and Pearson 1958). The assumption of a point dipole, on which equation (**7**) rests, is not as exact as treating the water molecule as a triangle of point charges, as discussed above (see Eley and Evans 1938). The two treatments are thus not quite equivalent. Nevertheless, equation (**7**) is simply transformed as follows:

(i) Since $\mu_0+\mu_i=2\alpha e r_w \cos\theta/2$ (where α is the charge on each hydrogen atom) the first term, giving the energy of electrostatic attraction between the cation and the water molecules, is a function of $r_w/(r_{M^{m+}}+r_w)^2$ and the second term, giving the mutual repulsion between the dipoles, is a function of $r_w^2/(r_{M^{m+}}+r_w)^3$.

(ii) Since the moment induced in a conducting sphere of radius r_w by a field F is $r_w^3F=\pi F$, the third term, giving the contribution from polarization, is equal to $3r_w^3F^2=3r_w^3Z^2e^2/(r_{M^{m+}}+r_w)^2$. The fourth term, which is the contribution from van der Waals forces between the water molecules, is usually small, as is the change in this term for different values of $r_{M^{m+}}$.

It will be seen from Figure 1 that the changes in ΔH^0 obtained with different values of $r_{M^{m+}}$ will not be very far from linear with $1/r_{M^{m+}}$ over the range of ionic radii in which we are interested ($r_{M^{m+}}=0\cdot80-2\cdot2$ Å), since the three important terms in equation (**7**) vary in this way. A similar conclusion can be deduced from the Eley-Evans' equation (**6**), which for our purposes can be regarded as equivalent, provided it includes both the permanent and the induced dipoles and charges. This causes some limitations in the predictions to be discussed later.

If we wish to evaluate ΔH^0 in any particular case, a precise knowledge of f, p, q, r, L, and D in equations (**2**-**6**) is necessary, and for this, some assumed

model is required (Duncan and Kepert 1959). However, we are concerned here with the variation of ΔH^0 for a series of complexes as M^{m+} is varied, with constant N^{n-}, or vice versa. If the complexes of the series have similar structure

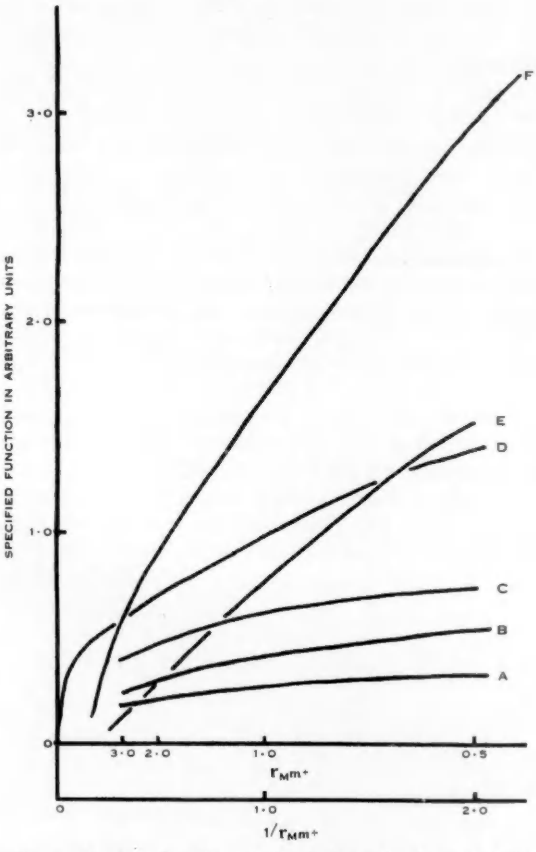


Fig. 1.—Various functions of the ionic radius ($r_{M^{m+}}$) plotted against $1/r_{M^{m+}}$.
A, $1/(r_{M^{m+}}+2r_w)$; *B*, $1/(r_{M^{m+}}+r_w)$; *C*, $\log\,[1/(r_{M^{m+}}+r_w)-1]$; *D*, $1/r^{\frac{1}{2}}_{M^{m+}}$; *E*, $1/(r_{M^{m+}}+r_w)^3$ ($\times 10$) ; *F*, $1/(r_{M^{m+}}+r_w)^2$ ($\times 10$) ; $r_{M^{m+}}$ and r_w ($=1\cdot 38$) are in angstrom units.

(e.g. similar coordination number, ionic charge, etc.) and all the above quantities are constant, then with the limitations already discussed, we may write

$$\Delta H^0 = C(r_{M^{m+}}) + D(r_{N^{n-}}) + J[1/(r_{M^{m+}}+r_w)] + W[1/(r_{N^{n-}}+r_w)] + X[1/(r_{M^{m+}}+r_{N^{n-}})] + Y[1/(r_{M^{m+}}+r_{N^{n-}}+2r_w)] + Z, \quad \ldots\ldots \quad (8)$$

where C, D, J, W, X, and Y are various functions of the quantities indicated, which are the only remaining variables, and Z is constant. This equation may now be used to predict the changes expected in ΔH^0 under a variety of conditions. Provided the entropy is constant, or negligible by comparison with the enthalpy change, or the entropy and enthalpy changes are proportional, these predictions will also be valid for the free-energy changes. Unfortunately, none of these conditions can in general be assumed. It is therefore necessary to discuss in some detail the impact of the entropy changes on the argument.

(*b*) *Entropies*

Entropy changes in the formation of $MN^{(m-n)+}$ will be made up of terms associated with (i) the movement and position of the water molecules and of M^{m+} and N^{n-} relative to each other (ΔS_w); (ii) the difference in energy of Born charging of the two separate ions and the species $MN^{(m-n)+}$ (ΔS_B); (iii) the difference in translational motion between (1) the species $MN^{(m-n)+}$ $[(S_T(g))_{MN^{(m-n)+}}]$ and (2) the two separate ions $[(S_T(g))_{M^{m+}}+(S_T(g))_{N^{n-}}]$ in the gas phase; and (iv) the difference in translational motion between (1) the species $MN^{(m-n)+}$ $[(S_T(aq))_{MN^{(m-n)+}}]$ and (2) the two separate ions $[(S_T(aq))_{M^{m+}}+(S_T(aq))_{N^{n-}}]$ in the aqueous phase. Hence,

$$\begin{aligned}\Delta S^0 = & (S_T(aq))_{MN^{(m-n)+}}-(S_T(aq))_{M^{m+}}-(S_T(aq))_{N^{n-}}\\ & -(S_T(g))_{MN^{(m-n)+}}+(S_T(g))_{M^{m+}}+(S_T(g))_{N^{n-}}\\ & +\Delta S_B+\Delta S_w. \qquad (9)\end{aligned}$$

We now consider each of these terms separately.

(i) *Relative Motion and Position of Participating Species.*—There are three types of motion to consider: (1) *Vibrational Entropy.* When a species $MN^{(m-n)+}(aq)$ is formed, from M^{m+} and N^{n-}, the following changes in vibrational entropy are possible:

(*a*) A gain due to liberation of hydration water molecules from both ions.

(*b*) A loss due to the vibration of the two ions relative to each other in the species $MN^{(m-n)+}$.

(*c*) A loss due to interaction of liberated water molecules with other water molecules.

Each of these entropy contributions is of the form

$$S_v = R[1+\ln(kT/h\nu)], \qquad (10)$$

where R is the gas constant, k is Boltzmann's constant, h is Plank's constant, and ν is the frequency of vibration. But even for highly charged ions, like Fe^{3+} (*a*) and (*c*) are less than 2·5 cal/degree/mole. The largest term is (*b*), which may be estimated by treating $MN^{(m-n)+}$ as a diatomic vibrator (Badger 1934, 1935), for which the force constant, $k_f = J^2/(r_{M^{m+}}+r_{N^{n-}}-d)^3$, where J and d are constants for species of the same general type. Hence,

$$\begin{aligned}S_v &= R[1+\ln\{2\pi kT(r_{M^{m+}}+r_{N^{n-}}-d)^{3/2}M^{\frac{1}{2}}_{M^{m+}}M^{\frac{1}{2}}_{N^{n-}}/hJ(M_{M^{m+}}+M_{N^{n-}})^{\frac{1}{2}}\}\\ &\approx K+\frac{3}{2}R\ln(r_{M^{m+}}+r_{N^{n-}}-d),\end{aligned}$$

where K is a constant for a given temperature and a series of similar species $MN^{(m-n)+}$ and M is the reduced mass of the appropriate hydrated ion.

(2) *Entropy of Movement of Hydration Water Molecules.* Although the water molecules which hydrate an ion are oriented at a fixed distance relative to a given cation, they may undergo motion in two directions about a sphere of diameter $r_{M^{m+}}+r_w$. The corresponding entropy contribution is

$$S_{M^{m+}}=pR\left\{\ln\left(\frac{2\pi kT}{h^2}\,\frac{m_w}{p}[4\pi(r_{M^{m+}}+r_w)^2-p\pi r_w^2]\right)+\ln p+1\right\}, \quad \ldots \quad (\mathbf{11})$$

for the cation, with a similar relation for the anion ; m_w is the mass of a water molecule. For the species $MN^{(m-n)+}$ we have

$$S_{MN^{(m-n)+}}=(p+q-s)R\left[\ln\left(\frac{2\pi kT}{h^2}\,\frac{m_w}{p+q-s}\pi\{2(r_{M^{m+}}+r_w)^2+2(r_{N^{n-}}+r_w)^2\right.\right.$$
$$\left.\left.+\sqrt{2}(r^2_{M^{m+}}+r^2_{N^{n-}})^{\frac{1}{2}}(r_{M^{m+}}+r_{N^{n-}}+2r_w)-(p+q-s)r_w^2\}\right)+\ln(p+q-s)+1\right],$$
$$\ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{12})$$

from which the total entropy change ΔS_m from this cause may be evaluated in conjunction with equation (**11**). Both ΔS_v and ΔS_m are small by comparison with ΔS_L, the libration entropy change.

(3) *Libration Entropy.* Owing to the hydration water molecules being in fixed orientation relative to the ions, their movement is restricted by comparison with water molecules in the bulk of the solvent. This, libration entropy, may be calculated by considering the partition function of the molecules in a field of force given by $V=-P\cos\varphi$, where P is the electrostatic energy. The total libration entropy change is

$$\Delta S_L=R\Sigma[\ln\{8\pi^2(8\pi^3I_1I_2I_3k^3T^3)^2/h^3\}-\ln(P/kt)]+\beta, \quad \ldots . \quad (\mathbf{13})$$

where I_1, I_2, and I_3 are the moments of inertia of the various species, and β is constant when a given number of hydration water molecules are replaced by N^{n-} or when N is varied, provided that the various P terms are always of the same type. The summation is taken, with appropriate sign, to include all species involved in the reaction, namely

$$MN^{(m-n)+}, \quad M\left(O\begin{matrix}\diagup H\\ \diagdown H\end{matrix}\right)^{n+}, \quad M\left(H-O\diagdown_{H}\right)^{n-},$$

and the interaction of liberated hydration water molecules with other water molecules in the bulk of the solvent, through both the O and the H atoms. Equation (**13**) is accurate for negative ions only at small displacements, but this is neglected here, since the error is small.

(ii) *Born Charging Entropy.*—This is $\Delta S_B=-\partial(E_B)/\partial T$, and is very small. It depends on $r_{M^{m+}}$, $r_{N^{n-}}$, and r_w in the same way as E_B.

(iii) *Translational Entropy in the Gas Phase.*—At 1 atm pressure and 25 °C, the translational entropy change in the gas phase may be derived from the equation

$$\Delta S_T(\mathrm{g}) = -\frac{3}{2}R\ln\{(pm_w+m_{\mathrm{M}^{m+}})(qm_w+m_{\mathrm{N}^{n-}})/m_w^s[(p+q-s)m_w+m_{\mathrm{M}^{m+}}+m_{\mathrm{N}^{n-}}]\}$$
$$-\frac{5}{2}(s-1)R\ln T+J', \qquad \ldots\ldots \quad (\mathbf{14})$$

where J' is a constant (see Latimer 1952) and the m's are the masses of the species indicated. This entropy contribution may often be of the same magnitude, but not of the same sign as the libration entropy.

(iv) *Translational Entropy in the Aqueous Phase.*—The entropy change arising from the difference in the translational entropy in the aqueous phase of the separate ions, M^{m+} and N^{n-} on the one hand, and the species $\mathrm{MN}^{(m-n)+}$ on the other is

$$\Delta S_T(\mathrm{aq}) = -\frac{3}{2}R\ln\{M_{\mathrm{M}^{m+}}M_{\mathrm{N}^{n-}}/M_{\mathrm{MN}^{(m-n)+}}\}+K, \qquad \ldots\ldots \quad (\mathbf{15})$$

where K is a constant.

(v) *Total Entropy Change.*—In evaluating the total entropy change, we have assumed that there is no change in the number of internal degrees of freedom when $\mathrm{MN}^{(m-n)+}$ is formed, except for vibrational motion associated with the bond M—N. This approximation is not always valid, especially when either M^{m+} or N^{n-} is a multidentate chelating group or ion. It is, however, quite acceptable when only simple monatomic ions are involved. Summing equations (**9–15**) we obtain for the total entropy change, at constant temperature

$$\Delta S^0 = \frac{3}{2}R\ln(r_{\mathrm{M}^{m+}}+r_{\mathrm{N}^{n-}}-d)+R'(r_{\mathrm{M}^{m+}}+r_w;\ r_{\mathrm{N}^{n-}}+r_w;\ r_w)$$
$$+Q[I_1I_2I_3;\ 1/(r_{\mathrm{M}^{m+}}+r_w);\ 1/(r_{\mathrm{N}^{n-}}+r_w);\ 1/(r_{\mathrm{M}^{m+}}+r_{\mathrm{N}^{n-}})]$$
$$+T[1/(r_{\mathrm{M}^{m+}}+r_w)]+U[1/(r_{\mathrm{N}^{n-}}+r_w)]+\frac{1}{D^2}\frac{\partial D}{\partial T}[1/(r_{\mathrm{M}^{m+}}+r_{\mathrm{N}^{n-}}+2r_w)]$$
$$+V(m_w;\ m_{\mathrm{M}^{m+}};\ m_{\mathrm{N}^{n-}})+\text{constant}. \qquad \ldots\ldots \quad (\mathbf{16})$$

The most important terms in this expression are those arising from ΔS_L, $\Delta S_T(\mathrm{g})$, and $\Delta S_T(\mathrm{aq})$, namely Q and V. The evaluation of these terms involves a knowledge of the moments of inertia of the various species involved, and their masses. Since neither of these quantities appears in the expression (**8**) for ΔH^0, ΔS^0 will not in general vary in the same way as ΔH^0. Nevertheless, under some conditions discussed in the next section one might expect a systematic relation between the entropy and enthalpy changes in the reaction.

(*c*) *Relation between Standard Thermodynamic Quantities*

Except where otherwise stated, we will consider M^{m+} to be the only independent variable. Three cases will be distinguished.

(i) ΔS^0 *Independent of* ΔH^0.—It is difficult to imagine a reaction in which ΔH^0 varies with $r_{\mathrm{M}^{m+}}$, whilst ΔS^0 remains unaltered. The condition implies that R, T, U, and F, and especially Q and V in equation (**16**) are unaltered by changes in $r_{\mathrm{M}^{m+}}$. But, although this can never be strictly true, the variation

in ΔS^0 may be insignificant when either $T\Delta S^0$ is negligibly small by comparison with ΔH^0, or when the different M^{m+} ions are very closely similar in ionic radius (e.g. for the rare earth ions). In such cases the moments of inertia variations, and the reduced mass variations may be small enough to neglect. The results of Fyfe (1952) for divalent metal amines appear to conform to this case. The values for ΔS^0 are quoted by him for fully ammoniated Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Mg^{2+}, which ions have different coordination numbers (4, 6, 4, 6, 2, and 4 respectively). Assuming the entropy changes per ammonia molecule to be the same for each one coordinating to a given cation, we get the following values of ΔS^0 per ammonia molecule : 4·06, 4·61, 3·45, 4·51, 3·85, and 4·66 cal/degree. Since the variation of ΔH^0 over the same ions is about 15 kcal/mole, it is clear that variations of ΔS^0 are only of the same order as experimental error, and can be neglected in the present context.

(ii) ΔS^0 *Varies Linearly with* ΔH^0.—This appears to be a common case in ion-pair formation (Duncan and Kepert 1959). It will only occur when Q and V in equation (**16**) vary with $r_{M^{m+}}$ in the same way as ΔH^0. This is not possible in the general case, since the terms in $1/(r_{M^{m+}}+r_{N^{n-}})$, $1/(r_{M^{m+}}+r_w)$, and $1/r_{M^{m+}}$ cannot be simply related. Nevertheless, if these quantities are plotted against each other, as in Figure 1, all of the quantities are found to be nearly linearly related to each other, over the range of values provided by Nature ($r_{M^{m+}}=0\cdot80$–$2\cdot2$ Å) even though the relations cannot be expressed as exact linear mathematical equations. The same is true for any quantity which is a reciprocal of $r_{M^{m+}}+\mathbf{S}$, where $\mathbf{S}$ is a constant of the same order as $r_{M^{m+}}$. Except for Q and V therefore, all the terms in equations (**6**) and (**16**) can be expressed as approximately linear functions of $1/r_{M^{m+}}$. In V, the masses of the ions are known not to be a simple function of the ionic radii, but to be effected by the commencement and completion of the electronic shells. Nevertheless, variations in V can be minimized by restriction of attention to a series of ions, for which $m_{M^{m+}}$ is either almost constant, or systematically depends on $1/r_{M^{m+}}$. It should also be noted that whereas the magnitude of V may be large compared with the other terms, the variation with $1/r_{M^{m+}}$ is small since the masses $M_{MN^{(m-n)+}}$, $M_{M^{m+}}$, and $M_{N^{n-}}$ are reduced masses (eqn. **15**)), and V is a logarithmic function. In Q, on the other hand, there may be large variations, arising from two causes. Firstly, there are changes in moments of inertia as M^{m+} is changed. The moments of inertia are complicated functions of the ionic radii, since they refer to the hydrated complex $MN^{(m-n)+}$, and the separated hydrated ions. The changes in ΔS^0 from this cause are therefore smaller than those from the electrostatic term, P (see eqn. (**13**)). This, the second cause of variations in Q, includes a contribution from the energy of interaction of M^{m+}. and N^{n-}. As with V, therefore, Q may in special cases be linear with $1/r_{M^{m+}}$, although one cannot expect it to be so in general.

From the previous discussion, one might expect ΔS^0 to vary systematically with ΔH^0 and $1/r_{M^{m+}}$ only in two cases (*a*) when Q predominates in ΔS^0 and changes in $r_{M^{m+}}$ are not sufficient to cause the moment of inertia changes to be serious (e.g. in the transition metal series), or (*b*) when V predominates in ΔS^0 and all ions are of the same electronic type (e.g. in the alkaline earths). When

the effects of Q and V are comparable, systematic relations might still be obtained, perhaps with some deviations. Unfortunately, not enough data are available to know how far the variations in ΔH^0 follow those of ΔS^0 and $1/r_{M^{m+}}$ in practice. Figure 2 shows two of the cases so far studied. In case A, the entropy contribution to the free-energy change is very large, and the enthalpy changes show no systematic dependence on $1/r_{M^{m+}}$.

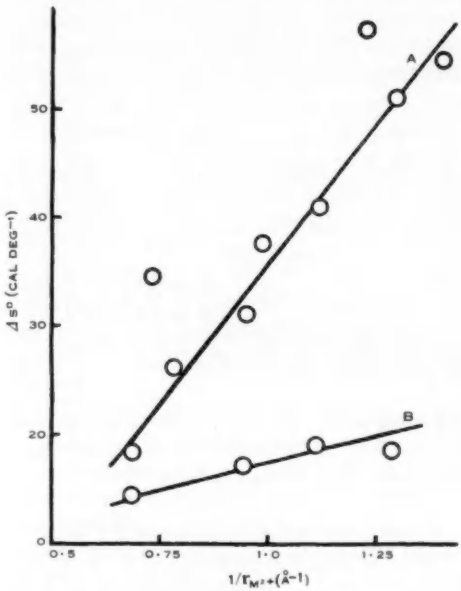


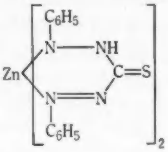
Fig. 2.—ΔS^0 plotted as a function of $1/r_{M^{m+}}$.

A, Ethylenediaminetetra-acetic acid (Charles 1954). Ions in order of increasing abscissa : Ba^{2+}, Pb^{2+}, Sr^{2+}, Ca^{2+}, Cd^{2+}, Mn^{2+}, Co^{2+}, Mg^{2+}, Ni^{2+}.

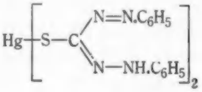
B, Nitrilotriacetic acid (Hughes and Martell 1954). Ions in order of increasing abscissa : Ba^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}.

(iii) *Other Possible Behaviour for* ΔS^0.—In some cases, reactions which are superficially similar may not in fact be directly comparable. The coordination number and stereochemical structure of the complex may not always be the same. Thus, in the transition metal ions, the coordination number of square planar Cu^{2+} is 4 (as in $Cu(CN)_4^{2-}$) compared with 6 for all other transition metal ions (e.g. $Mn(CN)_6^{4-}$). This may cause small differences in the entropy change of copper(II) complex formation, but the curves given below show that no gross differences arise for the formation of CuN^+ (N-monodentate) from that expected for the octahedrally coordinated transition metal ions. Apart from the geometrical arrangement of the bonds coordinated around the metal ion, however, structural differences may arise from other causes, viz. :

(*a*) *The Isomerization of the Ligand.* Thus, whereas for dithizone, most ions complex through the *N*-atom, as in



for some ions, notably mercury, bonding is apparently through the S atom, e.g.



(see below).

(*b*) *Ion-Pair Formation.* Whilst in barium sulphate the two ions are undoubtedly adjacent when they are paired, there is good evidence that in nickel sulphate the two ions are separated by at least one water molecule (Duncan and Kepert 1959 ; Eigen and de Maeyer 1959).

(*c*) *Steric Hindrance.* When steric hindrance is present the enthalpy-entropy relation may be anomalous. Irving and Rossotti (1956) have discussed cases such as 2-methyl-8-hydroxyquinoline where low stability constants for complex formation with large metal ions are ascribed to steric effects of the methyl group.

(*d*) *Theoretical Inadequacies.* In the theory it is assumed that the charges on the atoms which form the bond between the cation and the anion are integral. This is usually not the case, and it leads to some deviations which are discussed below.

Briefly, then, we may conclude that the entropy contribution to the free energy will not always vary in such a manner that it reflects the variation in the enthalpy. Sometimes one or both quantities will vary erratically with the ionic radius. Nevertheless, in the remainder of this paper we will assume that the free-energy changes vary in the same way as predicted for the enthalpy change—that is, we will consider only that experimental data in which there is *prima facie* evidence that the entropy changes are systematic with the enthalpy changes, or may be ignored. This is certainly not always a valid assumption. Subsequently, we shall consider some cases in which the predictions arrived at here are not fulfilled.

III. Application to Experimental Data

Many of the reactions experimentally studied so far proceed by stages, for which separate equilibrium constants are sometimes available, viz. :

$$M^{m+} + N^{n-} = MN^{(m-n)+} \qquad k_1, \qquad \ldots\ldots\ldots \quad (17)$$

$$MN^{(m-n)+} + N^{n-} = MN^{(m-2n)+} \qquad k_2, \qquad \ldots\ldots\ldots \quad (18)$$

$$MN^{(m-2n)+} + N^{n-} = MN^{(m-3n)+} \qquad k_3, \qquad \ldots\ldots\ldots \quad (19)$$

$$\cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \cdot$$

etc. up to k_z.

The arguments developed above may be applied with equal force to any of these association stages. Further, since

$$\Delta G^0 = \sum_1^z (\Delta G^0)_i = -RT \ln K_a, \quad \ldots\ldots\ldots\ldots \quad (20)$$

where ΔG^0 is the free-energy change for complete reaction, and K_a is the corresponding thermodynamic equilibrium constant, any conclusion relating to one stage of the reaction will also apply to the complete reaction unless one of the association stages introduces a new variable. In practice, overall stability constants are most easily experimentally accessible. Unfortunately, many of the quantities quoted in the literature are mass products, to which no correction has been applied for the activity coefficients of the various species present. This leads to errors, not only in the free energies, but also in the corresponding enthalpies and entropies. The values in the literature are therefore of variable uncertainty, as is confirmed by the wide range of values frequently reported by different authors for the stability constant referring to a particular reaction (see below). Being quite unable to correct for such errors we have accepted reported mass products as if they were thermodynamic stability constants. This causes some of the scatter in the diagrams presented below, but in general it is much less serious than the experimental uncertainties evident in the reported results of different workers.

When we apply the theoretical analysis to experimental data, we find that many of the plots which have appeared in the literature of free energy, enthalpy, and entropy against some other quantity are in fact intelligible, although often it is clear that they are not fundamentally sound quantities to use. The following variables may be discussed.

(*a*) *Ionic Radius*

It has been shown in Section II that the enthalpy change in complex formation is dependent on a number of functions of the ionic radius which, over the range of radii in which we are interested become closely linear with $1/r_{M^{m+}}$. Hence the enthalpy change, and (in cases where the entropy change is linear with the enthalpy change) also ΔG^0 will be linear with $1/r_{M^{m+}}$. In Figure 3 the thermodynamic quantities are plotted against $1/r_{M^{m+}}$ for a number of reactions with constant anions. Quite good straight lines are obtained for many of the ions. The slopes of the free-energy plots are not predictable, since they depend on the relation between ΔH^0 and ΔS^0. However, there is evidence (see Table 1) that the slope is largest for reactions resulting in strong covalent bonds. Ion pair and ion association reactions lead to slopes which are close to zero.

It should be noted that the Eley and Evans (1938) use of hydrated ionic radii and the Laidler (1956) and Latimer (1926, 1935, 1955) use of unhydrated (Pauling) radii can be reconciled if the difference in the energy of Born charging of the hydrated and the bare ion is approximately equal to the remaining energy terms in the expression for the hydration energy, of which the coordination energy is most important. This requirement is reasonable, since the transfer

of electrons from the water molecules to the cation must be equal (and of opposite sign) to the transfer of charge from the cation to the coordinated water shell of the hydrated ion. This, together with the fact that they are more readily accessible, justifies our use of unhydrated radii in the present context. We have used Goldschmidt radii here. These values have been criticized in some cases in that they refer to different valence states to those commonly found for the element concerned (e.g. the value for Zn^{2+} is calculated from the interatomic distances found in zincite (see Latimer 1952)). Such features account for some, but not all the scatter of data in the figures presented below. In particular, the ions Mg^{2+}, Cd^{2+}, Pb^{2+}, and Hg^{2+} occupy similar, but quite anomalous positions when the logarithms of the stability constants of complexes formed with a number of different anions are plotted as functions of $1/r_{M^{m+}}$ (see Fig. 3). This is due to differences in the screening by inner electrons of the effect of the nuclear field on the valence electrons for the above ions from that for the other ions quoted. An appropriate method of allowing for these differences is to estimate the effective nuclear charge ($Z_{\text{eff.}}$) by Slater's (1930) rules. The electrons in the valence shell of the anion cannot approach the cation closer than the innermost vacant electronic shell of the cation. It is, therefore, the effective charge of the cation relative to electrons in its valence shell which we require here. This is equivalent, in the molecular orbital model, to the assumption that the anion electrons fill the vacant orbital of the cation with lowest energy. The distance of separation between the cation and anion, however, is given by the sum of the ionic distances. We therefore plot in Figure 4, further thermodynamic data against $Z_{\text{eff.}}/r_{M^{m+}}$. $Z_{\text{eff.}}$ is taken from tables by Allred and Rochow (1958). Ions like VO^{2+}, TiO^{2+}, and also Cd^{2+}, Pb^{2+} now fall on the plot. The deviation of magnesium ethylenediaminetetra-acetic acid is only just greater than a reasonable experimental error. Hg^{2+} is anomalous in all cases, believed to be due to an erroneous value of $Z_{\text{eff.}}/r_{M^{m+}}$. A value of $5 \cdot 9$ would seem to be more reasonable, and would allow this ion to fit all the graphs which we have hitherto made in this way.

Figure 4 shows that provided allowance is made for the different electronic properties of the ions the scatter in the data is of the same order, but a little larger than experimental error in most cases. In part this may be due to differences in the polarizability of the ions, particularly of very large ions like mercury. The value of $r_{M^{m+}}$ appropriate to a particular cation will depend on the degree of polarization, and will therefore be slightly different for each compound. But this is a minor effect compared to crystal field stabilization. If, as is commonly believed, this contributes significantly to the stability constant, then it should be possible to estimate the magnitude of the contribution from the deviations of the ions from the line drawn through those ions for which crystal field stabilization is absent, namely Ca^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}, Mn^{2+}, and Hg^{2+}. The dotted line in Figure 4 is drawn through these ions. It will be noticed that even for these ions the scatter is almost as large as for the others. Nevertheless, we now estimate the crystal field stabilization for the remaining ions from the differences of $\log k_1$ from the values obtained on the dotted line for the same

values of $1/r_{M^{m+}}$. It should be noticed that even if the dotted line is drawn erroneously, the values of the crystal field energy of the ions will be in the same order, and with the same differences, provided that the correct line is parallel

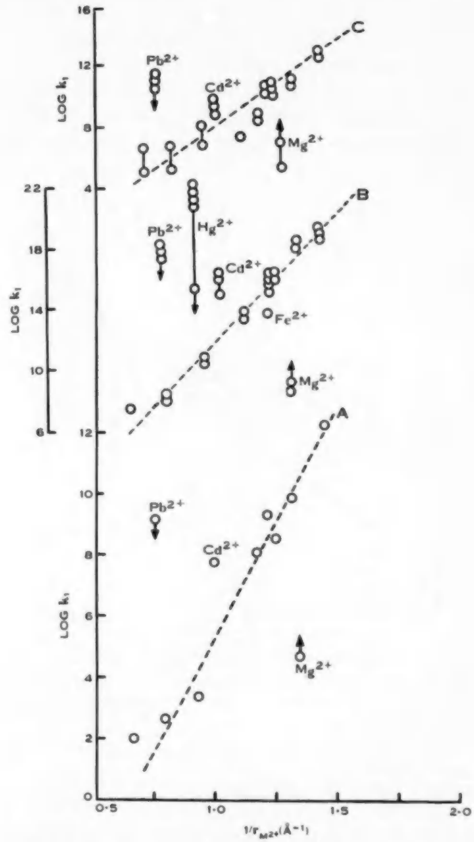


Fig. 3.—Log k_1 plotted as a function of $1/r_{M^{m+}}$ for (*A*) 8-hydroxyquinoline, (*B*) ethylenediaminetetra-acetic acid, and (*C*) nitrilotriacetic acid. Except for those marked, ions appear in the following order along the abscissa: Ba^{2+}, Sr^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+} (except *A*), Ni^{2+}, Cu^{2+}. Points with the same abscissa refer to different experimental data for the same ion (Stability Constants 1957).

to that drawn. The significance of the negative stabilization energy for Fe^{2+}, which undoubtedly arises from unsatisfactory experimental data, need not therefore concern us. We can directly compare these values with (1) those obtained from spectral data, if possible, and (2) values for a similar ligand for

which crystal field stabilization energy is known. Figure 4 (*D*) shows that some of the deviations in the curves of Figure 4 could in fact be attributed to crystal field stabilization, although it is clear that the experimental errors are almost as large.

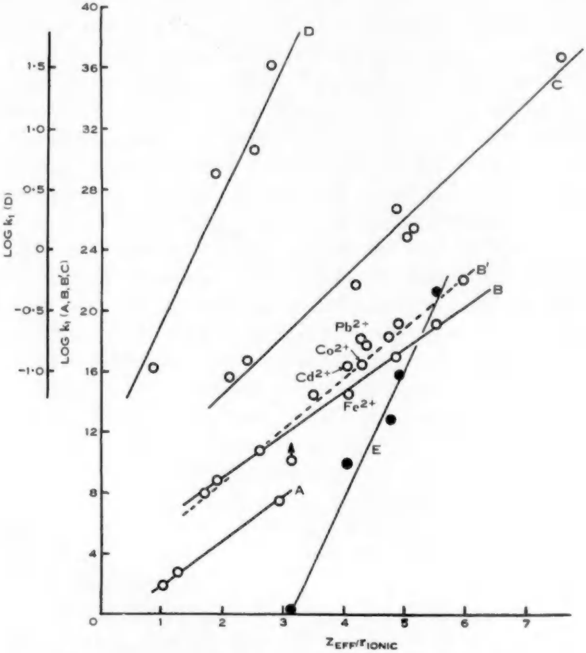


Fig. 4.—Log k_1 plotted as a function of $Z_{eff.}/r_{M^{m+}}$ for ethylenediaminetetra-acetic acid, in order along the abscissa, as indicated. $r_{M^{m+}}$ is in angstrom units.

A, Monovalent ions: H^+, Li^+, Ag^+.

B, Divalent ions: Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, TiO^{2+}, Ni^{2+}, Zn^{2+}, VO^{2+}, Cu^{2+}, (Hg^{2+}).

C, Trivalent ions, La^{3+}, Nd^{3+}, Ti^{3+}, V^{3+}, In^{3+}, Fe^{3+}, Co^{3+}.

- - - - - - - -

D, Log k_1 plotted as a function of the expected crystal field stabilization energy for the ions Fe^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}. The abscissa scale indicates kcal/mole.

E, ΔH^0 plotted as a function of $Z_{eff.}/r_{M^{2+}}$ from the data of Fyfe (1952) for amines (points ●). Ions in order along the abscissa: Mg^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}. The values of Ni^{2+} and Cd^{2+} have been reduced by one-third to allow comparison between these hexacoordinated ions and the tetracoordinated ions.

(*b*) *Quantities Related to the Ionic Radius*

The literature abounds with relations which purport to show that the free-energy changes in complex formation are dependent on other physical quantities such as ionization potential, hydration energy, etc. In fact, no fundamental significance can be attached to any of these relations. They arise because the

relation concerned is either itself dependent on the ionic radius, in the same way as the free-energy changes, or approximates to the same relation over the range of ionic radii normally considered. Examples are quoted below.

(i) *Hydration Energy* (*Fig. 5*).—The heat of hydration $(\Delta H_{M^{m+}})_H$ of a metal ion (Eley and Evans 1938) can be shown to be an approximately linear function of $1/r_{M^{m+}}$ by a similar method to that given above for complex formation. Hence, provided the coordination number and structure of the hydrated ion and metal complex remain the same throughout the series, $(\Delta H^0_{M^{m+}})$ and $(\Delta H_{M^{m+}})_H$ will be linearly related. It is difficult experimentally to determine $(\Delta H_{M^{m+}})_H$ with precision, and a wide scatter is usually obtained.

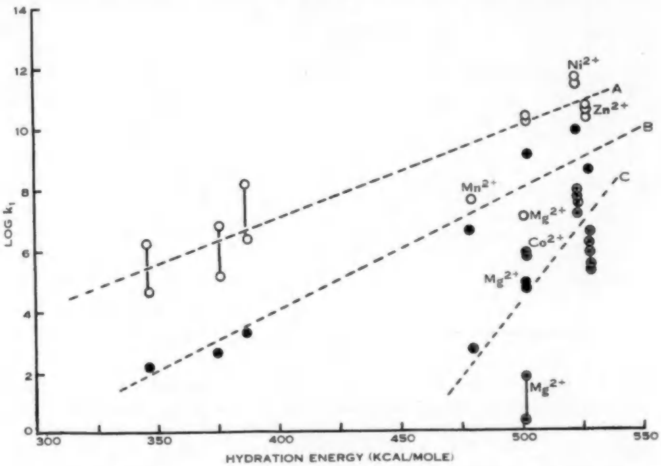


Fig. 5.—Log k_1 plotted as a function of the hydration energy $(\Delta H_{M^{m+}})_H$ for *A*, nitrilotriacetic acid (points ○); *B*, 8-hydroxyquinoline (points ●); *C*, ethylenediamine (points ⊙).

Ions in order along abscissa: *A* and *B*: Ba^{2+}, Sr^{2+}, Ca^{2+}. *A*, *B*, and *C*: Mn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}.

(ii) *Reciprocal of the Sum of the Ionic Radii of the Cation and Anion.* This type of plot is favoured by Nancollas (1956). But as seen from Figure 1, $1/(r_{M^{m+}}+r_{N^{n-}})$ is almost linear with $1/r_{M^{m+}}$ in all cases where $r_{M^{m+}}$ is less than about 2 Å. As with the other quantities discussed below, this must be regarded as a fortuitous result of the restricted range of ionic radii available in Nature.

(iii) *Ionization Potential.* It is quite common to use the second ionization potential as a function for systemizing free-energy data (see Freiser 1952). In general, however, convincing relations are only obtained when the ions are members of the same electronic series. Thus, the first long period transition metal complexes give reasonable straight lines (see Fig. 6), as would be expected, since the ionization potential (I_p) is (except for chromium) linear with $1/r_{M^{m+}}$ for this series. In general, however, the ionization potential depends critically on

the details of the electronic structure, such as the number of inner electronic shells, and whether s, p, or d electrons are involved in the bonding. Deviations of the ionization potential from linearity with $1/r_{M^{m+}}$ may then be of the order of 1000 kcal/mole, indicating that this is a quite unsatisfactory quantity for relating to free-energy changes in complex formation.

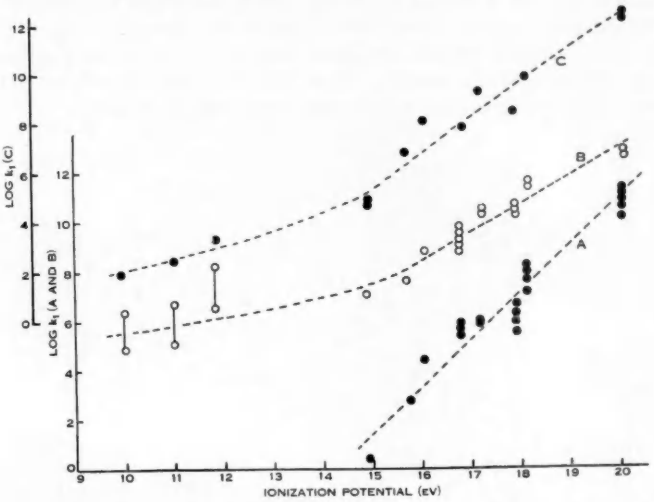


Fig. 6.—Log k_1 plotted as a function of the second ionization potential for A, ethylenediamine; B, nitrilotriacetic acid; C, 8-hydroxyquinoline. Ions in order along abscissa: B and C only: Ba^{2+}, Sr^{2+}, Ca^{2+}. A, B, and C: Mg^{2+}, Mn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}. Points with the same abscissa refer to different experimental data for the same ions (Stability Constants 1957).

(iv) *Standard Enthalpy of Formation of the Aqueous Ion* $[H^0_{M^{m+}(aq)}]$. From the cycle

$$\begin{array}{ccc} M(s) & \xrightarrow{-H^0_{M^{m+}(aq)}} & M(aq) \\ \Big\downarrow{\scriptstyle -E_s} & & \Big\uparrow{\scriptstyle (\Delta H_{M^{m+}})_H} \\ M(g) & \xrightarrow{-I_p} & M^{m+}(g)+e \end{array}$$

we get

$$H^0_{M^{m+}}(aq) = E_s + I_p - (\Delta H_{M^{m+}})_H. \quad \ldots\ldots\ldots\ldots \quad (21)$$

Again, although $(\Delta H_{M^{m+}})_H$ is linear with $1/r_{M^{m+}}$, the terms E_s and I_p prohibit $H^0_{M^{m+}}$ also being linear in the general case. But since the change from element to element in I_p is usually much greater than that of E_s, then linear relations can be obtained for log k_1 plotted both against $H^0_{M^{m+}}$ and against $G^0_{M^{m+}}$ provided that the same restrictions are used as apply to I_p above (see Fig. 7).

(v) *Standard Enthalpy of Formation of the Metal Complex.* For reactions of the type (**1**) we may in general write

$$\Delta H^0 = H^0_{\mathrm{MN}} - H^0_{\mathrm{M}^{m+}(\mathrm{aq})} - H^0_{\mathrm{N}^{n-}(\mathrm{aq})}$$
$$= (H^0_{\mathrm{MN}} - H^0_{\mathrm{HN}}) - (H^0_{\mathrm{M}^{m+}(\mathrm{aq})} - H^0_{\mathrm{H}^+(\mathrm{aq})}) + (H^0_{\mathrm{HN}} - H^0_{\mathrm{H}^+(\mathrm{aq})} - H^0_{\mathrm{N}^{n-}(\mathrm{aq})}).$$

Hence

$$\Delta H^0 = \Delta H^0_{\mathrm{MN}} - H^0_{\mathrm{M}^{m+}(\mathrm{aq})} - \Delta H^0_{\mathrm{HN}}, \quad \ldots\ldots\ldots\ldots \quad (\mathbf{22})$$

where ΔH^0_{MN} is the standard enthalpy of MN relative to that of the undissociated acid HN, $H^0_{\mathrm{M}^{m+}(\mathrm{aq})}$ is the standard enthalpy of M^{m+}(aq) relative to that of the aqueous hydrogen ion in its standard state—that is, it is the normally tabulated standard enthalpy of M^{m+}(aq), and ΔH^0_{HN} is the enthalpy change of the reaction

$$\mathrm{H^+(aq) + N^-(aq) = HN(aq)}.$$

From equation (**22**) it follows that since ΔH^0_{HN} is independent of M^{m+} and if both ΔH^0 and $H^0_{\mathrm{M}^{m+}(\mathrm{aq})}$ are linear functions of $1/r_{\mathrm{M}^{m+}}$, then ΔH^0_{MN} will also be a

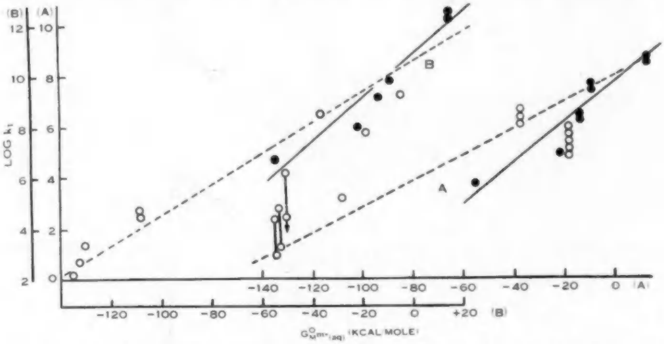


Fig. 7.—Log k_1 plotted as a function of $G^0_{\mathrm{M}^{m+}(\mathrm{aq})}$ for (*A*) nitrilotriacetic acid and (*B*) 8-hydroxyquinoline. Ions in order along abscissa: Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+} (*B* only), Cu^{2+}. The heavy lines are drawn through the transition metal ions (points ●).

linear function of $1/r_{\mathrm{M}^{m+}}$, and of ΔH^0 and $H^0_{\mathrm{M}^{m+}(\mathrm{aq})}$. A similar relation holds for free energies. In Figure 8 we plot ΔG^0_{MN} against $G^0_{\mathrm{M}^{m+}(\mathrm{aq})}$ for a number of cases. It will be noticed that the scatter in the points is much less in this case than in Figure 7. This is because both ΔH^0_{MN} and $H^0_{\mathrm{M}^{m+}(\mathrm{aq})}$ can be expressed as $f(1/r_{\mathrm{M}^{m+}}) + E_s + I_p$. Hence, any variations in sublimation energy and ionization potential which cannot be expressed as $f(1/r_{\mathrm{M}^{m+}})$ merely cause the plotted points to be moved along the line, whereas they cause deviations, which are often large in plots of ΔH^0 against $H^0_{\mathrm{M}^{m+}(\mathrm{aq})}$. The slopes of the lines in Figure 8 are not necessarily unity. They are determined by the dependence of the terms in equation (**22**) on the electrostatic terms discussed in Section I. There is evidence that the slope of ΔG^0 against $G^0_{\mathrm{M}^{m+}(\mathrm{aq})}$ becomes larger, the more tightly bound is the complex $\mathrm{MN}^{(m-n)+}$ (Duncan and Kepert 1959; see also Table 1).

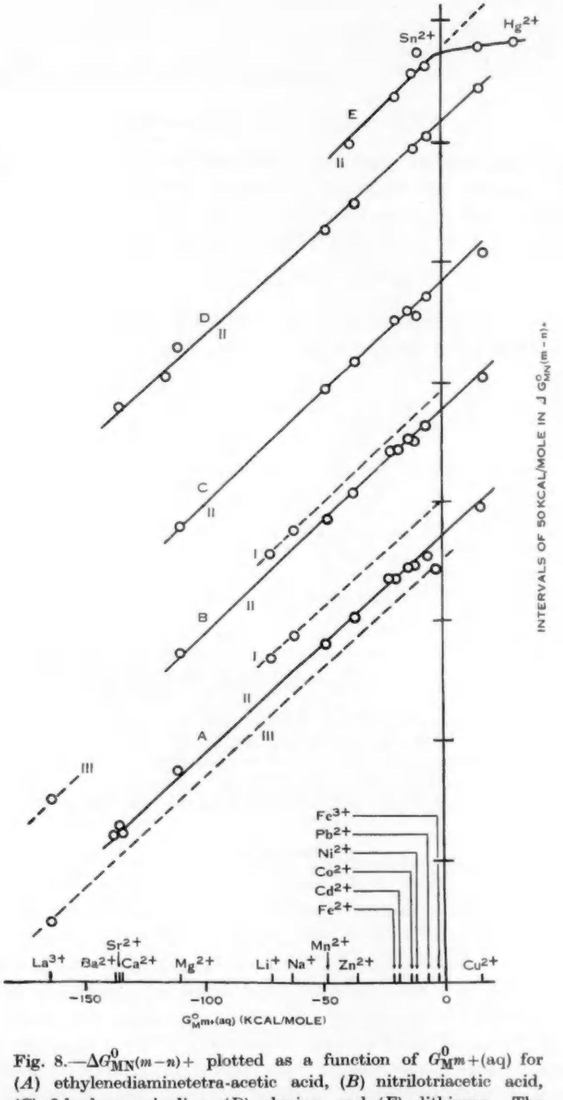


Fig. 8.—$\Delta G^0_{\mathrm{MN}(m-n)+}$ plotted as a function of $G^0_{\mathrm{M}m+}$(aq) for (*A*) ethylenediaminetetra-acetic acid, (*B*) nitrilotriacetic acid, (*C*) 8-hydroxyquinoline, (*D*) alanine, and (*E*) dithizone. The ionic charge is indicated by the symbols I, II, and III. The ions are indicated along the abscissa. The intervals along the ordinates are the origins for the plots closest to them.

TABLE I

SLOPES OF PLOTS OF LOG k_1 VERSUS $G^0_{M}m+(aq)$ FOR A NUMBER OF COMPLEXES

Y, ethylenediaminetetra-acetic acid ; D, 8-hydroxyquinaldine ; Ox, 8-hydroxyquinoline ; S, salicylaldehyde ; Nt, nitrilotriacetic acid

Complex	Slope per Bond	Reference to Data	Complex	Slope per Bond	Reference to Data
HgX_4^{2-}	$0\cdot25\pm0\cdot03$	Sillen and Laljequist (1944, 1949)	MS^+ (in 50% dioxane)	$0\cdot022$	Stability Constants (1957)
HgX_3^-	$0\cdot33\pm0\cdot04$		$M(COO)_2$	$0\cdot014\pm0\cdot002$	
HgX_2	$0\cdot35\pm0\cdot05$		$MCH_2(COO)_2$	$0\cdot012\pm0\cdot006$	
HgX^+	$0\cdot45\pm0\cdot05$		MOH^+	Close to zero†	Bell and Panckhurst (1956)
$M(en)^{2+}$	$0\cdot15\pm0\cdot02$	Mellor and Maley (1948)	MSO_4^-	$0\cdot0\pm0\cdot006$	Harned and Owen (1950)
$(M(NH_3)_4)^{2+}$	$0\cdot09$	Latimer (1952)	MNO_3^+	Close to zero†	Righellato and Davies (1930)
MCl^{2+}	$0\cdot105$		$Co^{III}(NH_3)_5X^{2-}$	$0\cdot0\pm0\cdot01$‡	Latimer (1952)
MY^{2-}	$0\cdot09$*	Stability Constants (1957)	MCl^+	$0\cdot0\pm0\cdot01$	
MD^+	$0\cdot044\pm0\cdot004$		TlX^{2+}	$0\cdot022$	Bell and George (1953)
MS^+	$0\cdot044\pm0\cdot01$		ZnX^+	$-0\cdot07$‡	Sillen and Laljequist (1944, 1949)
MOx^+	$0\cdot041\pm0\cdot01$		$Co(NH_3)_6{}^+X^-$	Negative, close to zero $+0\cdot075$‡	Evans and Nancollas (1953)
MNt^+	$0\cdot04\ \pm0\cdot02$		$Co(en)_3^{3}+X^-$	Negative, close to zero $+0\cdot075$‡	
MOx_2	$0\cdot012\pm0\cdot002$				

* For the reaction $M^2+Y^{4-}=MY^{2-}$.
† Data badly scattered.
‡ From H^0.

(c) Comparison of Thermodynamic Quantities for Two Different Anions

Suppose we have a series of cations M^{m+} which associate with one of two anions, P^- and Q^- to form $MP^{(m-1)+}$ and $MQ^{(m-1)+}$. Equation (**22**) will apply to each type of association. Hence, if $(\Delta H_a^0)_{MP^{(m-1)+}}$ and $(\Delta H_a^0)_{MQ^{(m-1)+}}$ are the enthalpies of association of the first stages, then

$$(\Delta H_a^0)_{MP^{(m-1)+}} + (\Delta H_a^0)_{MQ^{(m-1)+}} = (\Delta H^0_{MP^{(m-1)+}} - \Delta H^0_{MQ^{(m-1)+}}) - (\Delta H^0_{HP} - \Delta H^0_{HQ}), \quad \ldots\ldots\ldots\ldots\ldots\ldots \; (\mathbf{23})$$

and

$$\Delta H^0_{MP^{(m-1)+}} - \Delta H^0_{MQ^{(m-1)+}} = AH^0_{M^+(aq)} + B, \quad \ldots\ldots\ldots\ldots\ldots\ldots \; (\mathbf{24})$$

where A and B are constants. If both $\Delta H^0_{MP^{(m-1)+}}$ and $\Delta H^0_{MQ^{(m-1)+}}$ give lines of the same slope when plotted against $H^0_{M^+(aq)}$, then A will be zero, and $(\Delta H_a^0)_{MP^{(m-1)+}}$ will be linear with $(\Delta H_a^0)_{MQ^{(m-1)+}}$ with unit slope, and an intercept determined by the difference between the heats of formation of HP and HQ. Irving and Rossotti (1956) have given numerous illustrations of similar relations between free energies. But such relations will not be obtained if the dependences of $(\Delta H^0)_{MP^{(m-1)+}}$ and $(\Delta H^0)_{MQ^{(m-1)+}}$ on $H^0_{M^+(aq)}$ are different. In particular, if the charges on P and Q are not the same or, if, in the case of the complex, the fractional charges on the atoms adjacent to M^{m+} are different in the two cases, a linear relation cannot be expected to hold. Hence, it will not be obtained for complexing agents of different structure, when the coordinating atoms are different elements, or (in the case of polydentate complexing agents) of different number. Nevertheless, in cases where it is valid, quite good correspondence between $(\Delta G_a^0)_{MP^{(m-1)+}}$ and $(\Delta G_a^0)_{MQ^{(m-1)+}}$ is often obtained, close enough for reliable predictions of unknown stability constants to be made, Figure 9 (A/B) shows examples; Figure 9 (C/D) shows examples of the many non-linear relations obtained for complexes of different chemical structure.

(d) Comparison of the Thermodynamic Quantities for Two Different Cations and a Series of Anions

If we have a series of anions N^{n-} which associate with two cations L^+ and M^+ to form $LN^{(n-1)-}$ and $MN^{(n-1)-}$, we may write

$$(\Delta H_a^0)_{LN^{(n-1)-}} - (\Delta H_a^0)_{MN^{(n-1)-}} = \Delta H^0_{LN^{(n-1)-}} - \Delta H^0_{MN^{(n-1)-}} - (H^0_{L^+(aq)} - H^0_{M^+(aq)}), \quad \ldots\ldots\ldots\ldots \; (\mathbf{25})$$

which expression is constant, provided that the slope of the lines obtained when $\Delta H^0_{LN^{(n-1)-}}$ is plotted as a function of $H^0_{L^+(aq)}$ are the same for all N. Again, this is only possible if the different N have the same charge, or fractional charge etc., and both L and M are of the same charge. Irving and Rossotti (1956) quote one of the very few examples of this type for free energies.

(e) Special Cases

In conclusion, we give below some special types of behaviour which might be obtained even when the entropy and enthalpy changes are linear with each other.

(i) *Coordination Number and Structure.*—If any of the anions in a series has structural features which are different from the remainder, it will appear as an anomaly. An example is dithizone, for which the plot of $\Delta G^0_{ML_2}$ *v.* $G^0_{M^{2+}(aq)}$ deviates seriously from linearity. In Figure 8, values of $K_c p_c$ for the metal dithizonates, quoted by Pilipenko (1950, 1953) have been used to deduce apparent values of $\Delta G^0_{ML_2}$. K_c is the mass product for the formation of the neutral complex, and p_c is the extraction coefficient between water and the solvent used for determining the mass product. The assumption that p_c is constant for all metal

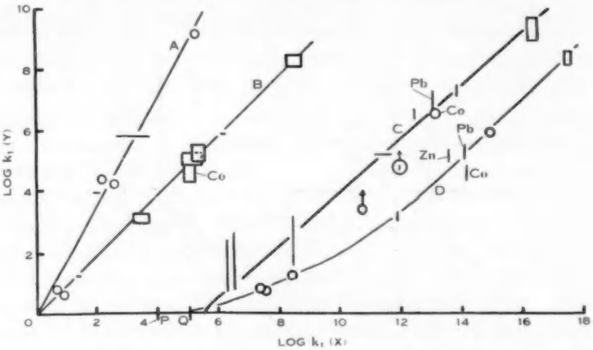


Fig. 9.—Log k_1 for two complexing agents plotted against each other.

Curve :	*A*	*B*	*C*	*D*
X-Scale :	Acetic acid (5*X*) ;	glycine, as indicated ;	8-hydroxyquinoline; (*X*+4, origin *P*)	8-hydroxyquinoline; (*X*+5, origin *Q*).
Y-Scale :	Glyceric acid ; (5*Y*).	alanine, as indicated ;	nitrilotriacetic acid, as indicated ;	alanine, as indicated.
Ions in order of increasing *X* :	Ba^{2+}, Sr^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+} ;	Ba^{2+}, Sr^{2+}, Ca^{2+}, Mn^{2+},(Co^{2+},Zn^{2+}), Pb^{2+},Ni^{2+},Cu^{2+} ;	Ba^{2+}, Sr^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+}, (Pb^{2+}, Co^{2+}),Ni^{2+},Cu^{2+} ;	as in curve *B*.

The range of values quoted in the literature is indicated by lengths of line drawn parallel to the appropriate axis. For points drawn in circles, only one value is available for each complexing agent, and no experimental errors are quoted (see Stability Constants 1957).

dithizonates may cause variations in $\Delta G^0_{ML_2}$ of perhaps 1 kcal/mole, which is of the order of experimental error. By comparison, it will be seen that $\Delta G^0_{ML_2}$ for copper dithizonate is too low by about 13·5 kcal/mole, and the mercury complex by about 35 kcal/mole. This indicates that these metals form complexes of quite different type, compared with those of the other ions. In the case of mercury, coordination appears to take place through the sulphur atom (Duncan and Thomas, unpublished data) rather than through the nitrogen atoms, as occurs in the case of nickel. Copper is also anomalous, but the reason is not necessarily the same as for mercury.

(ii) *Stepwise Equilibria.*—On the model discussed above, one would not expect the energy of chelation of one ligand to a metal ion to be the same as another ligand of the same type coordinated to the same ion, even if they were in equivalent structural positions. Experimentally this is often found to be the case, a fact which is often taken to be statistical in origin—the second ligand has one fewer coordination position which it can occupy than the first coordinated ligand. Such features would cause differences in the entropy contributions to the free-energy changes, but they would not affect the enthalpies. Nevertheless, on account of the electrostatic repulsion of the ligands for each other, enthalpy differences would be expected. The differences may be evaluated by assuming

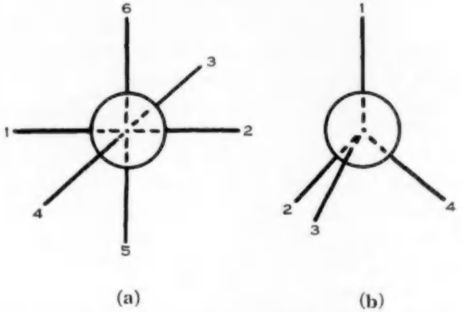


Fig. 10.—Stereochemical labelling for (*a*) octahedral (1–6) and square planar (1–4) complexes; (*b*) tetrahedral complexes.

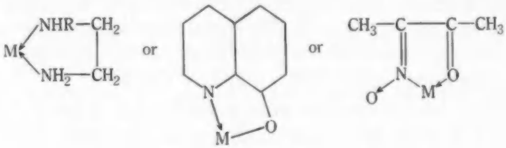
that substitution takes place in the order 1–6 in Figure 10 (*a*) for octahedral complexes, and 1–4 for square planar complexes (Fig. 10 (*a*)) and tetrahedral complexes (Fig. 10(*b*))—that is, the successive ligands are as spatially separated as possible. If the electrostatic attraction of all ligands to the central metal ion are the same, the differences between the enthalpies of attaching successive ligands to the ion will be determined by the electrostatic repulsion between the ligands. If Δ_{rs} refers to the enthalpy differences between the rth and the sth ligand, a simple analysis will show that we should expect the relation

$$\Delta_{12}=\Delta_{34}$$

to hold for all types of complex. For the octahedral type, Δ_{56} will have the same value. We should not expect *a priori* that a similar relation will hold for free energies, but when entropy differences are small, such a relation would be reasonable. In Table 2, we quote some results to show how frequently the theoretical expectations are in fact obtained.

Treatment of bidentate ligands in these terms is more difficult for two reasons: (*a*) the entropy contribution to the last step in which complex formation is completed is likely to be significantly different from that associated with the first and second steps. (*b*) The electrostatic repulsion terms in the various steps are not simply related to each other. One would not expect, therefore,

to obtain simple relations between the corresponding stability constants. Likewise one would not expect complexes such as



to have related free energies of formation, even when the same types of atom are adjacent to the cation. To obtain linear relations between the thermodynamic properties one must compare a series of complexes of similar basic structure, for example, those formed by differently substituted ethylenediamines.

(iii) *Dielectric Constant*.—From equations (**8**) and (**16**) it follows that the free energy of formation is dependent on the dielectric constant of the medium. If the coordination energy variations are greater than those from the Born charging energy terms, then increase of dielectric constant will cause a decrease in the free energy of formation. This is found to be the case for proton complexes of O-coordinated ligands (Irving and Rossotti 1956) in dioxan. The stability constants increase with increasing percentage dioxan in water–dioxan mixtures (decreasing D). But owing to the fact that the Born charging energy varies with D in the opposite sense to the coordination energy, the stability constant may sometimes

TABLE 2

DIFFERENCES BETWEEN STABILITY CONSTANTS FOR A NUMBER OF METAL COMPLEXES

Data from Stability Constants (1957)

Ligand	Metal Ion	Expected Stereo-chemistry	Conditions	$\frac{\Delta_{12}}{2\cdot 3RT}$	$\frac{\Delta_{34}}{2\cdot 3RT}$
Methylamine	Cd^{2+}	Octahedral	2·15M $NaNO_3$, 25 °C	0·68	0·52
Glyoxaline	Cu^{2+}	Square planar	0·16M $NaNO_3$, 25 °C	0·37	0·37
	Zn^{2+}	Tetrahedral	0·16M $NaNO_3$, 24 °C	—0·21	—0·21
	Cd^{2+}*	Octahedral	Ionic strength = 0·15, 25 °C	0·70	0·36
				0·59	0·42
				0·70	0·59
	Ni^{2+}		0·135M KCl, 25 °C	0·53	0·69
			0·16M $NaNO_3$, 25 °C	0·59	0·50
5(4) - Methyl glyoxaline	Cu^{2+}	Square planar	0·15M KNO_3, 25 °C	0·68	0·68
Pyridine	Cu^{2+}		0·5M KNO_3, 25 °C	0·66	0·46
			$C_5H_5N.HNO_3$, 25 °C	0·53	0·54

* The data for the cadmium glyoxaline complex refer to different determinations.

decrease with increasing D. This appears to occur with proton complexes of N-coordinated ligands (Irving and Rossotti 1956). Since N is a smaller atom (i.e. the electrostatic coordination terms are larger), this observation must be associated with the differences in the Born charging energies of the dipole $H^+—OH_2$ and the protonated N-complex, which would be expected to be rather larger than that between the $H^+—OH_2$ and an O-complex. It follows, therefore, that for metal complexes, variations with solvent composition may either increase or decrease, depending on the relative values of the terms involved.

(iv) *Variable Valency*.—Ions of different valency do not fall on the same plots (see Figs. 4 and 8). In general, the species $MN^{(m-n)+}$ is more stable the higher the charge on M. This is as expected, since the charge determines (*a*) the energy of Born charging, (*b*) the electrostatic energy, and (*c*) the crystal field stabilization energy. It is interesting to note that in Figure 8 the difference in intercepts between the lines for $Z=1$ and $Z=2$ is almost identical with that between $Z=2$ and $Z=3$, that is, $\Delta G^0_{MN^{(m-n)+}}$ is apparently proportional to $m-1$ for constant n, and $G^0_{M^{m+}}$. This could not have been predicted, but it reflects the higher binding energies obtained with higher Z, or (in Fig. 4) with smaller ionic radius for the same $Z_{\text{eff.}}$ for the neutral atom. The use of this dependence allows predictions to be made of the stability constants of complexes of ions like Th^{4+} and Ce^{4+}.

IV. Conclusions

It has been shown that an electrostatic interpretation of cation-anion association affords a convenient means of understanding many of the variations in stability constant experimentally observed. The conditions under which wide or spasmodic fluctuations are likely to be obtained can be predicted. There are however some systems (especially those in which the entropy changes do not reflect the enthalpy changes in complex formation), where discussion in terms of these concepts merits the introduction of special features which will be considered elsewhere.

V. Acknowledgments

Thanks are due to Mr. F. G. Thomas, who constructed some of the free-energy plots for the dithizone systems, and redetermined some of the values for this complexing agent quoted in the literature.

VI. References

Allred, A. L., and Rochow, E. G. (1958).—*J. Inorg. Nucl. Chem.* **5**: 264.

Badger, C. M. (1934).—*J. Chem. Phys.* **2**: 128.

Badger, C. M. (1935).—*J. Chem. Phys.* **3**: 710.

Ballhausen, C. J. (1954).—*Dansk. Mat. Fys. Medd.* **28** (4): 1.

Ballhausen, C. J. (1955).—*Dansk. Mat. Fys. Medd.* **29** (8): 1.

Basolo, F., and Pearson, R. G. (1958).—"Mechanism of Inorganic Reactions." p. 48. (Wiley & Sons: New York.)

Bell, R. P., and Panckhurst, M. H. (1956).—*J. Chem. Soc.* **1956**: 2836.

Bell, R. P., and George, J. H. B. (1953).—*Trans. Faraday Soc.* **49**: 619.

Born, M. (1920).—*Phys. Z.* **1**: 45.

Charles, R. G. (1954).—*J. Amer. Chem. Soc.* **76**: 5854.

DUNCAN, J. F., and KEPERT, D. L. (1959).—" The Structure of Electrolytes." Ch. 25, p. 380. (Wiley & Sons: New York.) (Proceedings of conference May 1957 at Washington, arranged by The Electrochemical Society.)

EIGEN, E., and DE MAEYER, L. D. (1959).—" The Structure of Electrolytes." Ch. 5, p. 64. (Wiley & Sons: New York.) (Proceedings of conference May 1957 at Washington, arranged by The Electrochemical Society.)

ELEY, D. D., and EVANS, M. G. (1938).—*Trans. Faraday Soc.* **34**: 1094.

EVANS, M. G., and NANCOLLAS, G. H. (1953).—*Trans. Faraday Soc.* **49**: 363.

FREISER, H. (1952).—*Analyst* **77**: 830.

FYFE, W. S. (1952).—*J. Chem. Soc.* **1952**: 2023.

HARNED, H. S., and OWEN, B. B. (1950).—" The Physical Chemistry of Electrolytic Solutions." (Reinhold Publishing Corp.: New York.)

HUGHES, V. L., and MARTELL, A. E. (1956).—*J. Amer. Chem. Soc.* **78**: 1319.

IRVING, H. M. N. H., and ROSSOTTI, H. (1956).—*Acta Chem. Scand.* **10**: 72.

JORGENSEN, C. K. (1954).—*Acta Chem. Scand.* **8**: 1502.

JORGENSEN, C. K. (1955*a*).—*Acta Chem. Scand.* **9**: 116.

JORGENSEN, C. K. (1955*b*).—*Acta Chem. Scand.* **9**: 1362.

LAIDLER, K. J. (1956).—*Canad. J. Chem.* **34**: 1107.

LAIDLER, K. J., and COUTURE, A. M. (1956).—*Canad. J. Chem.* **34**: 1209.

LATIMER, W. M. (1926).—*J. Amer. Chem. Soc.* **48**: 1234.

LATIMER, W. M. (1935).—*Chem. Rev.* **18**: 348.

LATIMER, W. M. (1952).—" The Oxidation States of the Elements." (Academic Press Inc.: New York.)

LATIMER, W. M. (1955).—*J. Chem. Phys.* **23**: 90.

LATIMER, W. M., and BUFFINGTON, R. M. (1926).—*J. Amer. Chem. Soc.* **48**: 2297.

MELLOR, D. P., and MALEY, L. (1948).—*Nature* **161**: 437.

NANCOLLAS, G. H. (1956).—*J. Chem. Soc.* **1956**: 744.

PILIPENKO, A. (1950).—*Zh. Anal. Khim.* **5**: 14.

PILIPENKO, A. (1953).—*Zh. Anal. Khim.* **8**: 286.

RIGHELLATO, E. C., and DAVIES, C. W. (1930).—*Trans. Faraday Soc.* **26**: 592.

SILLEN, L. G., and LALJEQUIST, B. (1944).—*Svensk Kem. Tidsk.* **56**: 85.

SILLEN, L. G., and LALJEQUIST, B. (1949).—*Acta Chem. Scand.* **3**: 539.

SLATER, J. C. (1930).—*Phys. Rev.* **36**: 57.

STABILITY CONSTANTS (1957).—" Stability Constants." Pt. 1. (Chemical Society: London.)

THE INFLUENCE OF MONOLAYERS ON EVAPORATION FROM WATER STORAGES

IV. THE SPREADING OF MONOLAYERS FROM SOLIDS

By W. W. Mansfield*

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Summary

It is shown that the nature and the rate of spreading of a monolayer from a solid depends upon the state and extent of the solid source and upon the hydrodynamic resistance to spreading. Experimental data describing spreading from solid cetyl alcohol are presented.

I. Introduction

In a preceding paper (Part III of this series, Mansfield 1959) it was shown that a monolayer spread upon an open water surface loses material continuously, so that an appropriate influx of new material is needed for its maintenance. When the monolayer is composed of cetyl alcohol or a similar substance, new material may be supplied either by spreading from solid or from a solution of the solid in a suitable solvent.

Whatever method be used, the rate of spreading may vary with the nature, extent, and disposition of the sources. The rate determines the degree to which losses from a monolayer are compensated and therefore influences the extent to which evaporation of water is reduced by the monolayer. Accordingly the design of a process for reducing evaporation necessitates a knowledge of the factors which control the spreading of monolayers on exposed water surfaces. As this knowledge is derived mainly from laboratory experiments, a clear understanding of the experiments is required in order to ensure that the results are used correctly.

Simple experiments show that spreading from cetyl alcohol (and many other similar compounds) may follow any one of three distinct processes. The most commonly-observed type of spreading is characterized by a well-defined boundary moving across the water surface and away from the spreading source, which is usually powdered solid. The movement may be followed most simply by dusting talc or a similar powder upon the surface. Even if the spreading is along a uniform channel of water, the boundary may move either at a constant, or at a variable, velocity. In this paper these processes are termed spreading at constant velocity and spreading at variable velocity respectively. Additionally, in some circumstances spreading develops without an easily-defined monolayer boundary, and is detected and measured by surface pressures. This process has been termed spreading at low surface pressures.

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II. Spreading at Low Surface Pressures

A single, smoothly-surfaced pellet (or but a few pellets) of cetyl alcohol placed on a water surface liberates molecules to the surrounding surface. Initially these molecules are distributed rapidly and evenly over the entire surface by surface flow and surface diffusion. For this circumstance the rate of transfer of molecules from the solid to the water surface is determined by the nature and extent of the perimeter of the solid and is defined by

$$\mathrm{d}N/\mathrm{d}t = Kp(F_e - F), \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where K molecules dyne^{-1} sec^{-1} is a constant dependent only upon the condition and temperature of the solid surface, p cm is the perimeter of the solid–liquid–air boundary, F_e dyne cm^{-1} is the equilibrium surface pressure generated by the solid, and F dyne cm^{-1} is the surface pressure of the water surface. Cary and Rideal (1925) found that relation (**1**) describes the spreading from a myristic acid crystal under certain conditions.

At low surface concentrations, monolayers of cetyl alcohol exert small surface pressures, which are independent of monolayer concentration and which are termed surface vapour pressures. For cetyl alcohol the surface vapour pressure, F_v dyne cm^{-1}, is about 0·1 dyne cm^{-1} at 25 °C (Adam 1941), and higher surface pressures are not recorded until the concentration of the monolayer reaches a limiting concentration, n_0, of $4 \cdot 56 \times 10^{14}$ molecules cm^{-2} (Nutting and Harkins 1939).

Accordingly the rate of release of molecules from a single, smooth-surfaced pellet of cetyl alcohol remains constant for some time. Using a surface pressure meter sensitive to about 0·2 dyne cm^{-1}, the spreading produces no detectable change of surface pressure, however, until a certain time has elapsed. The induction period, in sec, is given by

$$\tau = n_0 A / Kp(F_e - F_v), \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where A cm^2 is the area of the water surface.

Figure 1 shows that relation (**2**) is obeyed. From the slope of the plot, the rate of spreading of pure cetyl alcohol into a surface vacuum at (23 ± 1) °C is

$$K = (2 \cdot 42 \pm 0 \cdot 11) \times 10^{12} \text{ molecules dyne}^{-1} \text{ sec}^{-1}.$$

After the elapse of the induction period, relation (**1**) cannot describe the spreading of cetyl alcohol, as exploration of the surface reveals a gradient of surface pressure. A coherent monolayer is formed about the pellet and advances slowly across the surface. The transition to this type of spreading arises whenever the concentration within the annular layer surrounding the source exceeds the limiting concentration, n_0 molecules cm^{-2}. If the perimeter of the spreading source is increased greatly relative to the perimeter of the initial surrounding monolayer, such an advancing monolayer may be formed immediately.

There are some substances which do not form condensed monolayers even at their equilibrium pressure. Thus spreading from a myristic acid crystal at 25 °C follows relation (1) up to the equilibrium pressure, and not merely at very low pressures (Cary and Rideal 1925).

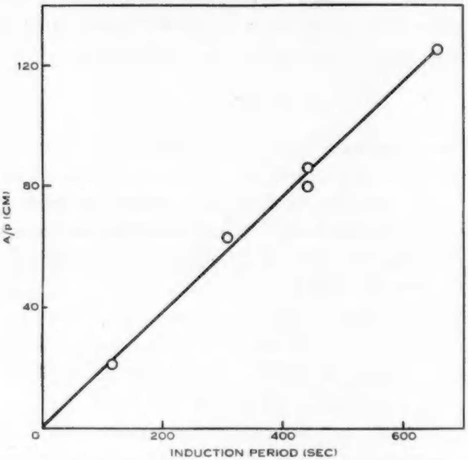

Fig. 1.—The influence of water surface area and of source perimeter on the spreading of cetyl alcohol at low surface pressures. Temperature (23 ± 1) °C.

III. Spreading at Constant Velocity

When sufficient cetyl alcohol is placed upon the surface of water confined within a long rectangular channel, the perimeter of the monolayer spreading along the channel is much less than the total perimeter of the spreading source. Thus spreading occurs as the growth of a visibly coherent monolayer, and is easily measured after dusting talc upon the surface.

When the channel is sufficiently wide, the curvature of the advancing front of the monolayer may be neglected, and the shearing stress imposed upon the already-covered section of the water surface is

$$f = F/l,$$

where F dyne cm^{-1} is the surface pressure of the monolayer which has travelled l cm.

The application of a surface stress to a channel of liquid produces surface flow. Keulegan (1951) has shown that, in the steady state, surface flow is balanced by return flow within the liquid, the return flow being maintained by a surface gradient. For this circumstance the surface velocity is

$$u_s = fz/4\eta,$$

where z cm is the depth of liquid of viscosity η poise.

A steady state cannot exist as a monolayer advances along a channel. If the hydrodynamic conditions are such that steady states are approached continuously as the monolayer advances, however, its approximate rate of advance is correspondingly

$$\mathrm{d}l/\mathrm{d}t = Fz/4\eta l. \qquad \text{(3)}$$

The conditions for which this approximation is valid are discussed in Section VII.

As the sources of spreading are surrounded by a monolayer of surface pressure F dyne cm^{-1}, the rate of release of molecules is

$$\mathrm{d}N/\mathrm{d}t = Kp(F_e - F),$$

and the velocity of the monolayer front is

$$\mathrm{d}l/\mathrm{d}t = Kp(F_e - F)/nw, \qquad \text{(4)}$$

where n molecules cm^{-2} is the concentration of the advancing monolayer and w cm is the width of the trough.

Comparing (3) and (4),

$$\frac{\mathrm{d}l}{\mathrm{d}t} = \frac{zF_e}{4\eta l(1 + nwz/4\eta lKp)}, \qquad \text{(5)}$$

and

$$F = \frac{F_e}{1 + nwz/4\eta lKp}. \qquad \text{(5a)}$$

When

$$pl/wz \ll n/4\eta K, \qquad \text{(6)}$$

these expressions simplify to

$$\mathrm{d}l/\mathrm{d}t = KpF_e/nw, \qquad \text{(7)}$$

and

$$F = 4\eta lKpF_e/nwz. \qquad \text{(7a)}$$

From (7a), the surface pressure of the advancing monolayer is always small. Accordingly the monolayer concentration may be set, with little error, at n_0 molecules cm^{-2}, the concentration at which the monolayer becomes homogeneous but has sensibly zero pressure. Thus

$$\mathrm{d}l/\mathrm{d}t = KpF_e/n_0 w, \qquad \text{(8)}$$

indicating that the monolayer spreads at a constant velocity which depends upon the ratio of the two perimeters. In Figure 2 it is shown that relation (8) is obeyed when the experimental conditions are apposite. From the slope of the plot, the spreading of cetyl alcohol into a surface vacuum at (24 ± 1) °C is

$$K = (2 \cdot 31 \pm 0 \cdot 07) \times 10^{12} \text{ mol dyne}^{-1} \text{ sec}^{-1}.$$

This value is lower than that reported in Section II, but is not significantly so. The agreement is sufficient to indicate that concordant values are obtained from the two quite different modes of spreading.

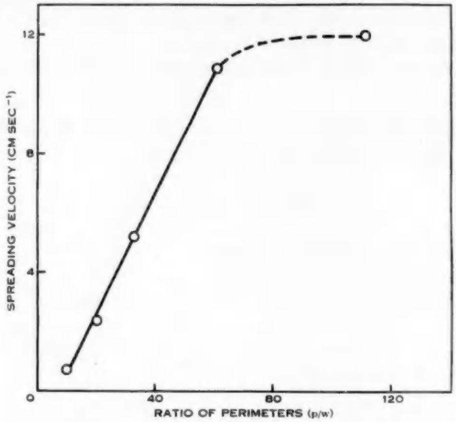

Fig. 2.—The spreading of cetyl alcohol at constant velocity. Temperature (24 ± 1) °C. Depth of water between 3 and 6 cm.

IV. Spreading at Variable Velocity

If a large quantity of finely-divided cetyl alcohol is placed upon water held in a long narrow trough, the condition

$$pl/wz \gg n/4\eta K \qquad \text{(9)}$$

must be satisfied after the monolayer has travelled a sufficient distance. Relations (**5**) and (**5a**) simplify to

$$\mathrm{d}l/\mathrm{d}t = zF_e/4\eta l, \qquad \text{(10)}$$

and

$$F = F_e. \qquad \text{(10a)}$$

Thus the monolayer, at the equilibrium surface pressure, advances at a velocity which increases with the depth of the channel but which decreases with the extent of travel.

Experiment shows that the surface pressure of a cetyl alcohol monolayer is close to 40 dyne cm^{-1}, the equilibrium pressure, when relation (**9**) is satisfied, and in Figure 3 it is shown that relation (**10**) describes the spreading of cetyl alcohol when the depth of the channel is near 0·7 cm. The relation is not satisfied for depths between 0·1 and 0·7 cm, where spreading rates are described approximately by

$$\mathrm{d}l/\mathrm{d}t = kzF_e/\eta l. \qquad \text{(11)}$$

In Figure 4 it is shown that k varies with the depth of the channel, but is not affected significantly by the length of travel of the monolayer.

If a monolayer spreads at the rate given by relation (**10**), there can be no net transport of liquid along the channel, as this is a condition of (**3**) from which (**10**) is derived. At the same time, there must exist a surface slope to promote the equalizing return flow, and this surface slope can be created only by the transport of liquid with the advancing monolayer. Thus relations (**3**) and (**10**) cannot accurately describe the spreading of monolayers as the conditions imposed are physically incompatible. Nevertheless deviations from the fully compensated flow described by (**3**) and (**10**) should become less significant as the depth of the channel is increased, as the relative amount of liquid transport required for the surface slope lessens. This trend is illustrated by the data of Figure 4.

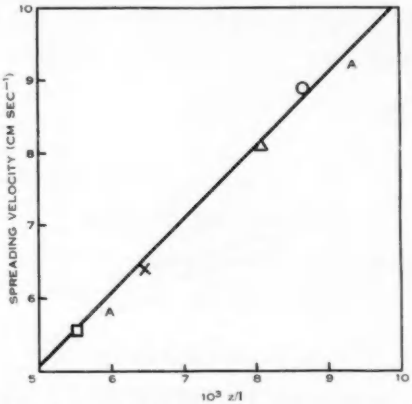

Fig. 3.—The spreading of cetyl alcohol at the equilibrium surface pressure. Temperature 21 °C. The line AA is estimated from relation (**10**). ○ Depth 0·68 cm ; × depth 0·71 cm ; □ depth 0·72 cm ; △ depth 0·76 cm.

V. The Transition to Compensated Flow

When a surface shearing stress f dyne cm^{-2} acts upon a channel of liquid of length l cm and depth z cm, fluid velocities u cm sec^{-1} at distances h cm from the bottom are established, together with a surface slope of $\mathrm{d}\gamma/\mathrm{d}l$. When flow is steady, and the surface displacements are small compared with the depth of water, the relation between the fluid velocities and the surface slope is (Keulegan 1951)

$$\frac{\mathrm{d}^2u}{\mathrm{d}h^2}=\frac{g}{\nu}\cdot\frac{\mathrm{d}\gamma}{\mathrm{d}l},$$

where g cm sec^{-2} is the gravitational acceleration and ν cm^2 sec^{-1} is the kinematic viscosity of the fluid. When the surface velocity is u_s cm sec^{-1}, this becomes

$$\mathrm{d}^2\theta/\mathrm{d}\beta^2=\alpha, \qquad \text{(12)}$$

where $\theta=u/u_s$, $\beta=h/z$, and $\alpha=(gz^2/\nu u_s)(\mathrm{d}\gamma/\mathrm{d}l)$.

For the boundary conditions $\theta=0$, $\beta=0$ and $\theta=1$, $\beta=1$, and $v=\int_0^1 \theta \mathrm{d}\beta$ the solution of (**12**) is

$$\theta=(3-6v)\beta^2+(6v-2)\beta. \qquad \textbf{(13)}$$

Thus

$$\alpha=6-2v.$$

Since $\alpha>0$, $v<\frac{1}{2}$. As v is a dimensionless measure of the flow per unit width across a vertical section of the channel, it cannot be negative. Hence

$$0<v<\tfrac{1}{2}.$$

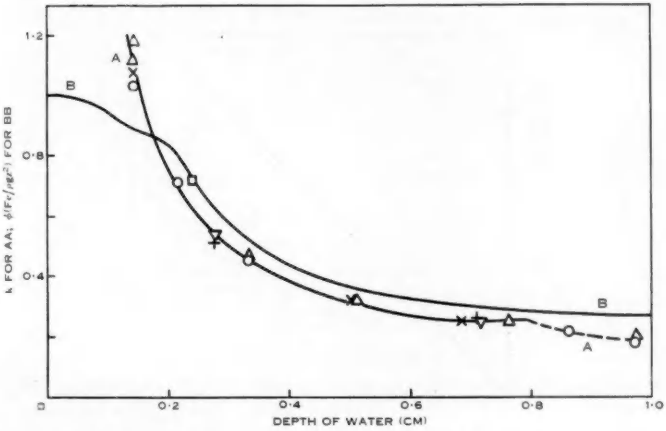

Fig. 4.—The influence of depth of water on the velocity of spreading of monolayers of cetyl alcohol. Temperature (20±2) °C. *AA* is fitted to the experimental data; *BB* is estimated from relation (**20**).
□ Mean extent 56 cm; ○ mean extent 72 cm; × mean extent 79 cm; △ mean extent 94 cm; + mean extent 110 cm; ▽ mean extent 130 cm.

The surface shearing stress is given by

$$f=\eta\,\frac{\mathrm{d}u}{\mathrm{d}h}=\frac{\eta u_s}{z}\,\frac{\mathrm{d}\theta}{\mathrm{d}\beta}. \qquad \textbf{(14)}$$

From (**13**) and (**14**),

$$u_s=fz/(4-6v)\eta, \qquad \textbf{(15)}$$

and the corresponding general relation for the advance of a monolayer is

$$\mathrm{d}l/\mathrm{d}t=Fz/(4-6v)\eta l. \qquad \textbf{(16)}$$

Thus the limiting relations are, remembering $0<v<\frac{1}{2}$,

$$\mathrm{d}l/\mathrm{d}t=Fz/\eta l,$$

and

$$\mathrm{d}l/\mathrm{d}t=Fz/4\eta l,$$

the first describing uncompensated flow and the second fully compensated flow.

For an advancing monolayer the rate at which fluid is transferred across a vertical section of the channel is

$$\mathrm{d}V/\mathrm{d}t = w\int_0^z u \ \mathrm{d}h$$
$$= wzv(\mathrm{d}l/\mathrm{d}t),$$

so that

$$\mathrm{d}V/\mathrm{d}l = wzv. \qquad (17)$$

But

$$\mathrm{d}V = wl \ \mathrm{d}\gamma,$$

giving

$$v = \frac{l}{z}\frac{\mathrm{d}\gamma}{\mathrm{d}l}. \qquad (18)$$

Keulegan (1951) has shown that in a channel of liquid subjected to a surface stress f dyne cm^{-2},

$$\mathrm{d}\gamma/\mathrm{d}l = (f + f_0)/\rho g z,$$

where $-f_0$ dyne cm^{-2} is the shearing stress at the base of the channel and ρ g cm^{-3} is the density of the liquid. With (**13**) and (**14**), this leads to

$$\mathrm{d}\gamma/\mathrm{d}l = (3 - 6v)f/(2 - 3v)\rho g z.$$

The corresponding relation when the shearing stress is provided by a monolayer is

$$\mathrm{d}\gamma/\mathrm{d}l = (3 - 6v)F/(2 - 3v)\rho g z l. \qquad (19)$$

From (**18**) and (**19**),

$$v(2 - 3v)/(3 - 6v) = F/\rho g z^2.$$

Thus v is a function only of $F/\rho g z^2$, and (**16**) becomes

$$\frac{\mathrm{d}l}{\mathrm{d}t} = \frac{Fz}{\eta l} \cdot \varphi(F/\rho g z^2), \qquad (20)$$

where φ is the appropriate function. Evaluation of the function shows that relation (**3**), describing fully compensated flow, is obeyed within 1 per cent. only if

$$F/\rho g z^2 < 8 \times 10^{-3}.$$

For spreading at constant velocity (Section III), this condition reduces to

$$pl/wz^3 < 4 \text{ cm}^{-2}$$

for the spreading of cetyl alcohol on water. The condition is approached for the experiments of Figure 2 which follow relation (**8**).

When the monolayer spreads at variable velocity (Section IV), the pressure of the monolayer is always close to the equilibrium spreading pressure. Thus, within the limits of the assumptions involved in deriving (**20**), the value of the function φ depends only on the depth of the liquid. For the spreading of cetyl alcohol upon water, relation (**10**) should be obeyed within 1 per cent. only if the depth of water equals or exceeds 2·26 cm. In Figure 4 the effect of depth determined experimentally is compared with the effect predicted by relation (**20**).

For small depths the assumption that liquid transport produces no significant changes in depth is invalid, and measured spreading velocities are greater than those predicted. As the depth is increased, compensated flow is approached more rapidly than is predicted by (**20**), and is sensibly attained at a depth of 0·7 cm. Beyond a depth of 0·8 cm, the rate is no longer inversely proportional to the extent of spreading, and the values of k given in Figure 4 were obtained by falsely assuming that it is. For these greater depths the liquid flow induced by the spreading monolayer is by no means laminar, and it is reasonable to assume that for intermediate depths a slight degree of turbulence aids the attainment of compensated flow.

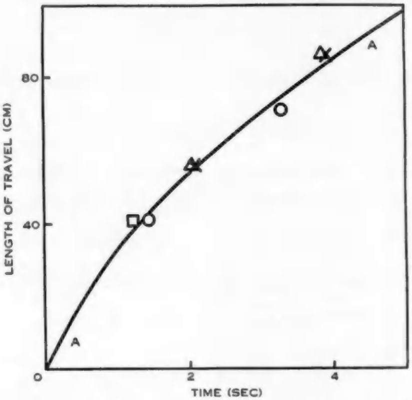

Fig. 5.—Spreading of cetyl alcohol monolayers when the induced liquid flow is turbulent. Temperature 23 °C. AA is calculated from relation (**21**). ○ Depth 3·3 cm; △ depth 4·8 cm; □ depth 5·8 cm; × depth 6·0 cm.

VI. The Influence of Turbulence

Keulegan (1951) has shown experimentally that fully compensated flow within a channel is laminar only if

$$u_s z/\nu < 600$$

approximately, and may be regarded as fully turbulent when

$$u_s z/\nu > 8000.$$

For depths of water exceeding about 0·8 cm, the spreading at variable velocity of monolayers of cetyl alcohol does not satisfy the condition for laminar flow at the range of travel (40–100 cm approximately) normally used in laboratory experiments. When the depth of water exceeds 3 cm, the condition appropriate for fully turbulent flow is met.

From Keulegan's data, the surface velocities obtained when flow is fully turbulent may be described approximately by assuming that all flow is laminar and within a layer of liquid immediately adjacent to the surface and of thickness

$$z' = 1690\nu/u_s.$$

Thus when spreading at the equilibrium surface pressure induces fully turbulent flow, the rate of spreading may be expected to be given by

$$\left(\frac{\mathrm{d}l}{\mathrm{d}t}\right)^2=\frac{1690F_e}{4\rho l}.$$

For cetyl alcohol on water, this reduces to

$$l=33\cdot 6t^{\frac{2}{3}}. \qquad \text{(21)}$$

In Figure 5 it is shown that this relation is followed when the experimental conditions are suitable.

VII. Discussion

In the preceding four sections it has been assumed that steady states of total flow within a channel are maintained continuously as a monolayer advances. As the conclusions based on this assumption are supported reasonably well experimentally, the assumption is approximately true under the conditions of the present experiments.

The author has been unable to obtain a completely satisfactory general description of the conditions for which the assumption is approximately correct. From Lamb (1945), however, the steady surface velocity found after λ sec when a constant shearing stress acts uniformly over a liquid surface, without producing surface slope, is given by

$$u/u_s=\psi(\nu\lambda/z^2),$$

where u_s cm sec^{-1} is the final velocity. Evaluation of the function ψ shows that the surface velocity is within 1 per cent. of the final velocity provided that

$$\nu\lambda/z^2>2.$$

By replacing λ sec by the average age of the advancing monolayer of cetyl alcohol on water, this requirement reduces to

$$l^2/z^3>2\cdot 4\times 10^6 \text{ sec}^{-1},$$

which is satisfied by those experiments of Figure 4 for which $k\sim 1$.

When $k<1$, it is probable that steady surface velocities are reached relatively more rapidly. For these surface velocities a surface slope is required, and whenever the surface velocity is less than u_s cm sec^{-1} water is transported and surface slopes created. On this basis, the requirement that observed velocities are within 1 per cent. of the steady velocity corresponding to $k=0\cdot 25$ is

$$l^2/z^3>1\cdot 2\times 10^4 \text{ sec}^{-1}$$

for the spreading of cetyl alcohol on water. This condition is satisfied for the appropriate experiments of Figure 4. It is reasonable to conclude that time effects do not sensibly influence the results of the investigation.

Both spreading at low surface pressure and spreading at constant velocity are controlled by the rate of release of molecules from the solid. Accordingly any measured spreading rate should depend upon the physical state, as well as

the chemical composition, of the spreading source. In this work reproducible values of the constant, K molecules dyne^{-1} sec^{-1}, were obtained only after exhaustive washing of the solid castings of cetyl alcohol with distilled water. Spreading rates measured with incompletely-washed surfaces were very erratic. Presumably during washing microcrystals are removed from the surfaces, but work by Mr. R. G. Vines (unpublished data) suggests that at the same time the crystalline phase of the cast material is standardized.

When spreading follows relation (**10**), the only surface property of the solid that affects the rate of spreading is the equilibrium surface pressure. As this property varies little with the state of the surface, virtually no control in preparation is needed to obtain reproducible data. Finally, when neither of the limiting conditions (**6**) nor (**9**) is applicable, reproducible measurements of spreading rates are to be expected only if both the surface characteristics of the solid source and the hydrodynamics of the channel are controlled.

It is clear that the nature and the rate of spreading of cetyl alcohol depend markedly upon the conditions available for spreading, and that both may change significantly during spreading from a source. The application of these conclusions to the control of spreading upon water storages is discussed in a following paper.

VIII. References

Adam, N. K. (1941).—"The Physics and Chemistry of Surfaces." 3rd Ed. p. 46. (Oxford Univ. Press.)

Cary, A., and Rideal, E. K. (1925).—*Proc. Roy. Soc.* A **109**: 301.

Evans, L. F. (1953).—*Aust. J. Appl. Sci.* **4**: 165.

Keulegan, G. H. (1951).—*J. Res. Nat. Bur. Stand. Wash.* **46**: 358.

Lamb, H. (1945).—"Hydrodynamics." 1st Amer. Ed. p. 592. (Cambridge Univ. Press.)

Mansfield, W. W. (1959).—*Aust. J. Appl. Sci.* **10**: 73.

Nutting, G. C., and Harkins, W. D. (1939).—*J. Amer. Chem. Soc.* **61**: 1180.

Appendix I

Experimental Methods

(*a*) *Spreading at Low Surface Pressures*

A quantity of purified cetyl alcohol (Evans 1953) was melted and poured into a glass tube, approximately 1 cm in diameter and sealed loosely at one end with a glass piston rod. After the melt had solidified the glass tube was warmed momentarily with hot water so that only material just in contact with the wall was remelted, allowing extrusion of about 1 cm of the casting. To allow easy handling, the extruded rods were left attached to the glass tubes. After thorough washing with distilled water, they were stored under distilled water.

A casting was lowered into a cleaned water surface defined by barriers placed across a surface trough, and at intervals thereafter readings of surface pressure were taken. Surface pressures were determined from optical magnification of the movement of a square glass coverslip dipping into the surface and attached to a beam and torsion wire. After some time the pressure rose sharply

and values of the induction period were obtained graphically by extrapolation to zero pressure. At the conclusion of each run the diameter of the casting was measured with a travelling microscope.

(b) *Spreading at Constant Velocity*

Molten cetyl alcohol was expelled from a glass capillary tip into a column of water. Hot (80 °C) water was led into the bottom of the column and cold (20 °C) water entered midway. Droplets of cetyl alcohol solidified as they ascended the column, were expelled with the waste water, and were collected upon a copper gauze. The beads were ellipsoidal and of mean diameter 2·6 mm. They were washed exhaustively with distilled water, dried between filter paper, and stored over water.

A few of the beads were floated upon water held in an optical cell, the walls of which previously had been rendered hydrophobic with a dilute solution of a cationic detergent. Images of the beads, at approximately 10 magnifications, were projected onto a ruled screen. Since elevated menisci were not formed at the walls of the cell, the floating level of the beads was delineated with reasonable precision. The mean spreading perimeter of the beads was estimated as 58·6 cm g^{-1}.

Spreading rates were measured on cleaned water surfaces in a copper trough 120 cm long, 8·3 cm wide, and 8 cm deep. At one end of the trough a little talc was dusted upon the water surface, so that the advancing boundary of the monolayer formed by a known weight of beads was quite well defined. The passage of the boundary between two perspex strips, placed 50 cm apart across the top of the trough, was timed with a stopwatch. The reflections of the strips at the water surface were used to avoid errors of parallax.

(c) *Spreading at Variable Velocity*

For most experiments spreading occurred from about 2 g of finely-ground, purified cetyl alcohol. For some runs, however, a ground sample of commercial cetyl alcohol of the same equilibrium spreading pressure was used. The two materials gave identical results.

Spreading rates were measured either in the trough 120 cm long, or in another 180 cm long. The number and the position of the perspex strips used varied from experiment to experiment. Liquid depths were measured with a glass pointer attached to a vernier movement reading to 0·01 cm.

PEROXIDES IN CARBON BLACK

By V. A. GARTEN* and K. EPPINGER*

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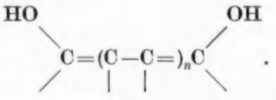
Summary

The varying ability of carbons to oxidize Fe^{II}, to produce H_2O_2 when exposed to air in an acid medium, and to form a latent photographic image, has been studied on a graded set of carbon blacks. Mechanisms are discussed for these three types of activity and it is shown how they agree with the phenol–lactone–chromene concept of carbon black chemistry.

I. INTRODUCTION

Chemical and infra-red spectroscopic evidence presented earlier (Garten, Weiss, and Willis 1957) suggested diminishing concentrations of lactones and phenols and increasing concentrations of chromenes in sugar chars and carbon blacks, when these are subjected to increasing temperatures in an inert atmosphere. The various types of lactones and the phenols were considered responsible for the acidic properties, and the chromenes for the alkaline properties of different carbons. The systematic approach to the problem of identification of the various chemical groups, applied to a series of carbons prepared from the same parent material, by subjecting it to specified treatments, proved extremely useful. A similar approach to the study of peroxide formation and to the detection of stable peroxidic structures in a selected carbon black reveals the operation of at least three different peroxidation mechanisms. One of these predominates in low-temperature carbons, and is obviously related to the presence, in these carbons, of relatively unstable hydroperoxide groups $\geqslant C-O-OH$ and of structures of the general type



A second operates in high-temperature carbons, preferably in an acid medium, involving chromenyl free radicals. A third mechanism results in the formation of peroxides, most likely of the transannular type, which represent a substantial part of the total oxygen content of all carbons and which are of relatively high stability.

Various workers (Yohe and Harman 1941; Jones and Townend 1950) have found that some carbons will oxidize Fe^{II}. Others (Burstein and Frumkin 1941; Winslow 1947) have shown that acid extracts of some carbons will react

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with permanganate; the fogging of photographic material, when placed near some carbonaceous substances in the dark, has also been known for a long time (Russel 1908; Haslam 1928).

An account is presented of a systematic study on peroxidation of a selected carbon black and it is shown how the data are in accordance with the phenol–lactone–chromene concept of carbon black chemistry.

II. Results

A summary of the results obtained with a series of carbons prepared from a high-colour carbon (which is characterized by a B.E.T. surface area of 700 m^2/g, a suspension pH of 3·9, and a C-content of 80 per cent.) by heating in pure nitrogen, as described earlier (Garten and Weiss 1955), is given in Figure 1. Carbons subjected to temperatures <600 °C produced no detectable hydrogen peroxide when in contact with acid in the presence of air; carbon heated to

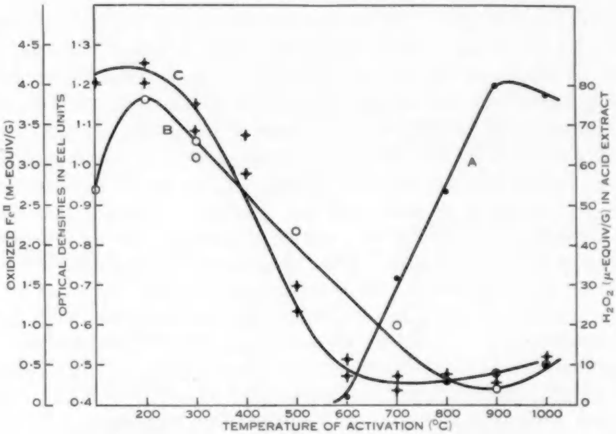


Fig. 1.—Concentrations of various peroxides as functions of the temperature of activation of the carbon black.

A, H_2O_2 (μ-equiv/g) in acid extract.
B, Oxidized Fe^{II} (m-equiv/g).
C, Optical densities in EEL units.

900 °C gave a maximum of 80 μ-equiv H_2O_2/g carbon (curve *A*). The concentration of transannular peroxides (curve *B*) decreases steadily with rising temperatures of activation from a peak concentration of more than 3 m-equiv/g appearing in the carbons heated to 200 to 300 °C. Finally, curve *C*, representing the optical densities produced by different carbons, which are considered to be due to the generation of hydrogen peroxide molecules from polyphenols and to the combined action of ·OH and ·OOH free radicals, indicates concentrations of these active species (expressed as H_2O_2) of the order of 1 to 10 μ-equiv/g.

The ability of the carbons to release hydrogen peroxide when in contact with acid and air (A) decreases considerably on prolonged exposure to the atmosphere, whereas the concentration of the transannular peroxides (B) remains unchanged. Fogging activity appears to decrease only partially probably owing to the loss of polyphenolic structures by a process of slow autoxidation.

III. Discussion

(a) *Frumkin Effect*

The mechanism proposed earlier (Garten and Weiss 1957), by which hydrogen peroxide is released by a carbon in contact with acid and air, requires some modification. First of all, the identity of hydrogen peroxide has been established beyond doubt by Burstein and Frumkin (1941) by means of the bichromate test, and also by the diminished stability in an alkaline medium and the complete destruction by catalase as observed in this laboratory. After extraction with acid, the washed or alkali-treated carbon regenerates part of its ability to react with fresh acid and oxygen. Furthermore, whereas the chromene concentration, determined by the method described earlier (Garten and Weiss 1957), remains unchanged on equilibrating freshly prepared samples in humid atmosphere, the reactivity towards acid and oxygen diminishes continuously and recovers only partially on heating to 70–100 °C. The whole of the available evidence, it seems, can be best explained by the presence in these carbons of resonating chromenyl sites rather than by isolated chromene groups. They can be envisaged as arising from the corresponding ground state by thermal excitation and they, thus, represent low-lying triplets. A very similar situation is met in low-temperature carbons, as will be discussed later. The changeover from one system of resonating microcircuits, as it were, in low-temperature carbons to another in high-temperature carbons, would, in the intermediate temperature range, leave some electrons unassociated and would thus account for the well-known fact of a maximum concentration of non-interacting free radical structures near 600 °C (cf. for example, Turkevich and Laroche 1958). In Figure 2, the reactions of dichromenyls with acid and oxygen, their possible regeneration by alkali and heat, and their conversion into dichromenols (pseudo-bases) by a process of slow autoxidation, are illustrated in some detail.

Several facts and observations lend support to the proposed scheme :

(i) Known examples of autoxidation of organic compounds with the simultaneous production of hydrogen peroxide invariably involve the abstraction of two equivalent hydrogen atoms or two electrons at a time, for example, from hydroquinones, hydrazobenzene, and various enediols such as ascorbic acid, benzoin, and others.

(ii) Regeneration or part regeneration of the Frumkin effect after acid extraction by thermal cleavage of ·OH free radicals from the alkali-treated carbon on renewed heating, is supported by the observed bleaching action of the regeneration process on a latent photographic image produced by light or heat.

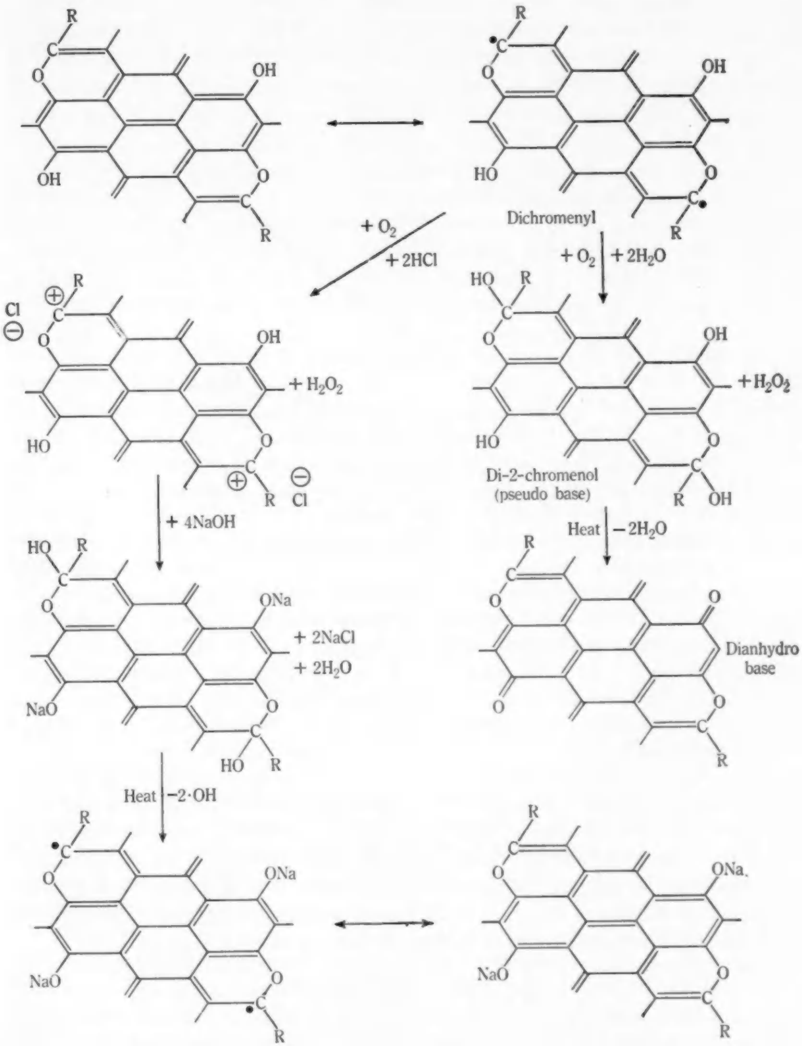


Fig. 2.—Some reactions of dichromenyls.

(iii) Without alkali treatment, the process of regeneration is less effective because of possible anhydro-base formation by intramolecular dehydration, involving both basic and acidic –OH groups in suitable positions.

(iv) Figure 3 indicates a near-unity ratio of equivalents hydrogen peroxide produced to the equivalents chromene estimated for the investigated series of carbons.

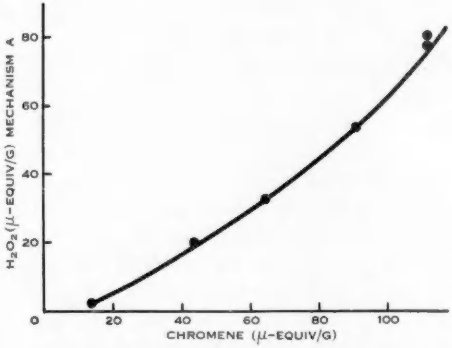
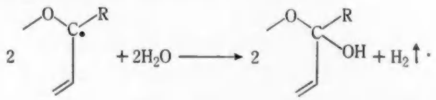


Fig. 3.—[H_2O_2] produced by carbon black (1 g) in the presence of acid and air *versus* [" chromene "].

(v) Mukherjee and Lahiri's (1957) experimental results on the low-temperature reaction of outgassed coals with water in which small amounts of hydrogen gas were shown to be produced, particularly by coals of high rank, at 100 °C would also be in agreement with the suggested mechanism, that is,



In the absence of oxygen, hydrogen gas is produced instead of hydrogen peroxide. This, in essence, represents a homolytic cleavage of water as already considered by Jones and Townend (1950).

The electron donating property of a chromenyl would agree with our knowledge of the behaviour of a tervalent carbon adjacent to oxygen and with the fact that it is easily converted into a carbonium ion.

(*b*) *Transannular Peroxides*

A second peroxidation mechanism (curve *B*, Fig. 1) has been studied by means of a modified Yule and Wilson (1931) technique, based on the oxidation of Fe^{II}. It is concerned with a type of peroxide present in concentrations nearly 30 times that of the two others. The peroxide responsible for this reaction must be considered stable in the range 200 to 300 °C or to reform immediately when a carbon, heated to these temperatures in an inert atmosphere, is re-exposed to

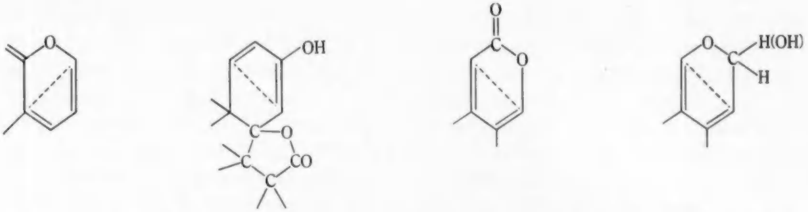
the atmosphere. It cannot be removed by evacuation or by a stream of dry argon, and it is regenerated completely by re-exposing the carbon to air after reduction with Fe^{II}. No loss in weight of the carbon occurs on 12 reduction-oxidation cycles. Reaction of the carbon with dienes, for example, butadiene or isoprene, but not with vinyl monomers such as styrene, methyl methacrylate, or acrylonitrile, followed by thorough washing with a suitable solvent, results in the loss of about 90 per cent. of its oxidation capacity, which it recovers, however, on continuous extraction with a solvent for 1 week. Oxidation of aqueous hydroquinone by the carbon, followed by thorough washing and drying of the carbon *in vacuo* at room temperature, increases its oxidation capacity by nearly 100 per cent., obviously due to the formation of quinone which remains strongly adsorbed on the carbon. Reaction of the carbon with lead tetra-acetate reveals only traces of oxygen gas evolved (optimum 10 μ-equiv/g), which rules out the possibility of hydroperoxides being responsible for the oxidation of Fe^{II}.

The reactivity towards dienes suggests the presence, in the carbons, of fixed double bonds which must be associated with the behaviour of the carbons to act as oxidants for Fe^{II} and hydroquinone. Barton, Smallwood, and Ganzhorn (1954) suggested that the oxidation of Fe^{II} by carbon was effected by quinone groups. Since the redox potential of a quinone depends on the presence of fixed double bonds, oxidation of Fe^{II} by the carbon could, at least partially, be due to quinones. These would, by reaction with a diene by way of a Diels-Alder mechanism, acquire a lower redox potential, no longer high enough to oxidize Fe^{II}.

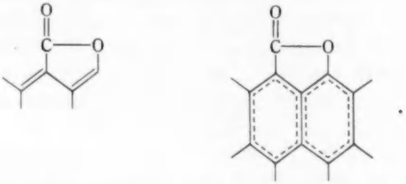
A quinone concentration of more than 3 m-equiv/g, however, can hardly be reconciled with the chemical evidence available. Low-temperature carbons may contain, at the most, 1 m-equiv/g of fluorescein-type lactones, which can be considered as pseudo-quinones (cf. Garten, Weiss, and Willis 1957). Studebaker *et al.* (1956) concluded from the increase in nitrogen on treating a high-colour carbon with diazomethane that, in this favourable instance, 1·6 m-equiv/g of quinone oxygen was present. Hallum and Drushel (1958), from polarographic studies, inferred the presence of quinones associated with peri-hydroxyl groups. The presence of these configurations, however, cannot be reconciled with the chemical evidence of methylation results (cf. Garten and Weiss in press). Furthermore, the oxidation of hydroquinone by the carbon would require the presence of quinone groups with redox potentials higher than that of benzoquinone.

It appears that the presence of transannular peroxides would justify all the chemical evidence if the concept of low-lying triplets in carbons were accepted. The formation of transannular peroxides in fully or partly aromatic compounds is obviously a Diels-Alder diene synthesis, in which oxygen plays the role of the dienophilic partner. It has been described for a number of 1,3-dienes by Moureu, Dufraisse, and Dean (1926*a*, 1926*b*), by Windaus and Brunken (1928), and particularly by Schenck and Ziegler (1944, 1951). Whereas, normally, trans-annular peroxides are formed by a process of photo-oxidation, releasing the absorbed energy in the form of chemi-luminescence on thermal decomposition,

an analogous mechanism may require much less energy to operate in carbons on suitable 1,3-diene structures, provided by the presence of ether, lactone, quinone, and chromene groups. Peripheral (sufficiently exposed) groups of this kind will all contribute to the stabilization of 1,3-dienes. Peripheral ethers, lactones of the fluorescein type, normal δ-lactones, and chromenes (or chromenols), as shown:

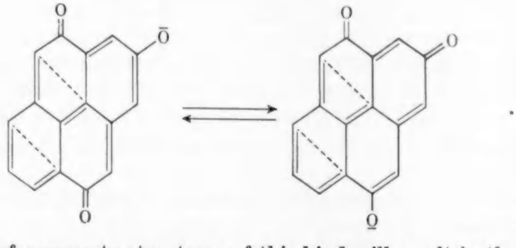


will provide sites for transannular peroxidation, whereas with γ-lactones, for example, a similar situation cannot be envisaged:



This would lend support to the earlier evidence (Garten, Weiss, and Willis 1957) favouring δ-lactones rather than γ-lactones.

Non-resonating quinones, if their presence in the low-temperature carbons can be established beyond doubt, would also, besides acting as oxidants for Fe^{II} in their own right, assist in the fixation of bonds and give rise to transannular peroxidation:



The attachment of oxygen to structures of this kind will result in the rearrangement of double bonds and will create new fixed olefinic bonds, which, in turn, would be available for reaction with suitable dienes, if activated, for example, by $>C=O$ groups in conjugation. The activation will greatly enhance the reactivity of the fixed double bonds towards a diene although, in some instances, even substances with isolated double bonds are known to react in the Diels-Alder fashion.

It could reasonably be expected that the concentration of this type of peroxide would bear a simple straight-line relation to the total oxygen content of the carbon (cf. Fig. 4), whereas a similar relation to the total moisture content of the carbon, as indicated in Jones and Townend's (1950) results, does not apply to the series of carbons investigated in the present work.

Since ordinary oxygen will also oxidize Fe^{II}, though at a much smaller rate, the transannular oxygen molecule, when leaving the free radical site, would have to be in some excited form. The existence, under certain conditions, of " active oxygen " has been considered by a number of workers (Kautsky 1931 ; Wittig and Lange 1938 ; Schenck 1948 ; Egerton 1949) but no final agreement has been reached.

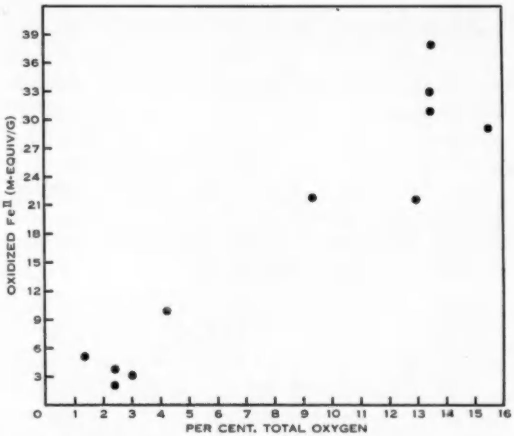


Fig. 4.—Oxidizing capacities for Fe^{II} *versus* total oxygen content of carbon blacks.

(c) *Russel Effect*

A third peroxidation mechanism (curve *C*, Fig. 1) operates in carbons prepared at temperatures <600 °C. The peroxide involved reveals itself by its reactivity towards lead tetra-acetate and by its ability to fog a photographic plate when kept in close proximity to it at 70 °C for 2 to 3 days. The photographic activity involves a volatile substance, and can be completely suppressed by impermeable screens. The effect can be greatly reduced by evacuation or by flushing and subsequent exposure in an inert gas. The fogging ability recovers on readmission of wet oxygen. The effect is greatly enhanced by the presence of a small amount of a ferrous salt and can be completely eliminated by treatment of the carbon with alkali. The activity can apparently be regenerated an infinite number of times by cooling an exposed sample in air and by renewed exposure.

Reaction with lead tetra-acetate is taken to be a reliable measure of hydroperoxides (Criegee, Pilz, and Flygare 1939 ; Hock and Lang 1942 ; Treibs 1944), the hydroperoxide groups being converted into ketones. The reaction is an

instantaneous one and produces an equivalent amount of oxygen gas. Unfortunately, the lead tetra-acetate reaction cannot be used quantitatively with carbon because after a short burst of gas evolution the reaction proceeds at a very slow rate with the production of mainly methane and CO_2 (total being approximately 90 per cent.). Only the initial rates are therefore given in Figure 5.

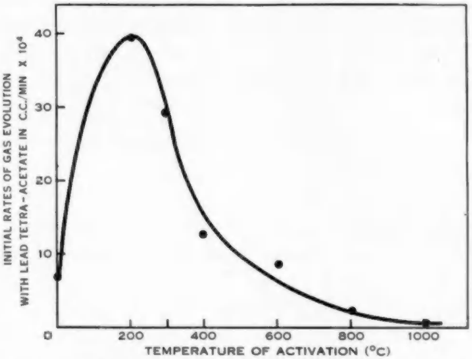


Fig. 5.—Initial rates of gas evolved with lead tetra-acetate from carbon black (5 mg).

The mechanism by which the hydrogen peroxide molecule fogs a photographic plate is not completely understood. Although a detailed discussion of the phenomenon, now widely known as the Russel effect, is outside the scope of the present paper, a few comments do not appear out of place. Whereas actinic action on a sensitive photographic emulsion results in the eventual complete transfer of an electron from a bromide anion to a silver cation, forming developable silver nuclei, we would suggest that the action of the hydrogen peroxide molecule is due to its twofold character as an oxidation-reduction agent. Thus, hydrogen peroxide first oxidizes a bromide anion, followed by a reduction of a silver cation by $\cdot OOH$ to metallic silver:

$$\begin{array}{c} Ag^+ + Br^- + H_2O_2 \rightarrow Ag^+ + Br\uparrow + OH^- + \cdot OH \\ \cdot OH + H_2O_2 \rightarrow \cdot OOH + H_2O \\ \cdot OOH + Ag^+ \rightarrow O_2 + Ag + H^+ \\ \hline Ag^+ + Br^- + 2H_2O_2 \rightarrow Ag + Br\uparrow + 2H_2O + O_2 \end{array}$$

or alternatively, hydrogen peroxide first reduces a silver cation, followed by an oxidation of hydrogen bromide by a further H_2O_2 molecule:

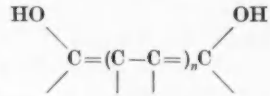
$$\begin{array}{c} Ag^+ + Br^- + H_2O_2 \rightarrow Ag + H^+ + Br^- + \cdot OOH \\ Ag^+ + Br^- + \cdot OOH \rightarrow Ag + H^+ + Br^- + O_2 \\ \hline 2AgBr + H_2O_2 \rightarrow 2Ag + 2HBr + O_2 \end{array}$$

and

$$2HBr + H_2O_2 \rightarrow 2H_2O + Br_2\uparrow$$

It will be noted that both schemes are based on the ability of hydrogen peroxide to act as an oxidant as well as a reductant. It is also apparent that, unless H_2O_2 molecules are available, which will, in the course of the reaction, produce both oxidizing ·OH free radicals as well as reducing ·OOH free radicals, a reducing action on the photographic plate can be produced only by ·OOH alone or in combination with ·OH, but not by the ·OH free radical species exclusively.

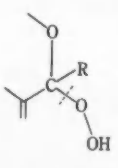
The very fact of regenerability of the Russel effect rules out structures of the general type:



as a persistent source of H_2O_2 production, although these may be involved at an early stage of peroxidation.

It appears that the problem of regenerability cannot be solved by assuming the presence of either phenols and enediols, or hydroperoxides of the more common type, as the latter invariably decompose by cleavage of the O–O bond and involve the irreversible conversion of the substrate $\geqslant$C–O–OH group into a >C=O group.

Taking again recourse to the concept of low-lying triplets in carbon, it would seem that in low-temperature carbons, with their high concentration of lactones (up to 1·5 m-equiv/g) and an appreciable concentration of phenols (up to 0·5 m-equiv/g), resonating circuits can be set up involving both carbon- and oxygen free radical sites. The two sites shown in Figure 6 are different in that the tervalent carbon site will have a distinctly reducing character, inviting the attachment of oxygen and forming a Ziegler-type addition compound $R \cdot O_2$. Ziegler and Ewald (1933) showed that compounds of this type can transfer up to 50,000 molecules of oxygen; also, according to Waters (1950), they are much stronger oxidants than ordinary oxygen. In the presence of further unpaired electrons in the system, a certain degree of polarization may result despite the fact that the electron would have to pass across the aliphatic carbon atom. This would explain the observed instantaneous quenching of the spin resonance by the admission of oxygen to an outgassed carbon. In the presence of moisture, hydroperoxides will form; these should decompose in the manner shown, as the C–O bond in the group will be weakened as follows from a consideration of electronegativity. In the liquid phase, ·OH and ·OOH would not persist for any length of time, reforming O_2+H_2O. In the gas phase, they may have some chance of meeting other partners before recombination. In essence, the proposed scheme suggests that on resonating free radical systems of this kind, oxygen and water can be fixed reversibly.



The operation of a Ziegler-type mechanism, which would not require the presence of water, is less likely as it would not explain the quenching action of alkali. Treatment of the carbons containing hydroperoxide groups with alkali will result in the formation of the corresponding sodium salts and will thus prevent the generation of ·OH and ·OOH. Alkali-treated carbon is photo-

graphically inactive. When the volatiles are allowed to pass through a small bed of crystalline ferrous sulphate, ·OH is eliminated, thus diminishing the possibility of reoxidation of previously formed silver nuclei. A small amount of Fe^{II} increases the photographic activity of these carbons considerably.

The concept of low-lying triplets in carbons, which has been used throughout the paper, appears, on available chemical evidence, more likely than that of the presence of genuine di-radicals with pairs of non-interacting single electrons. The latter may, however, exist in carbons prepared in the intermediate temperature range of approximately 600 °C. On the basis of the early experiments by Bonhoeffer, Farkas, and Rummel (1933) and by Rummel (1933) it is difficult to decide whether the activation energy of the hydrogen conversion in the range 0 to 200 °C is exactly zero or very small.

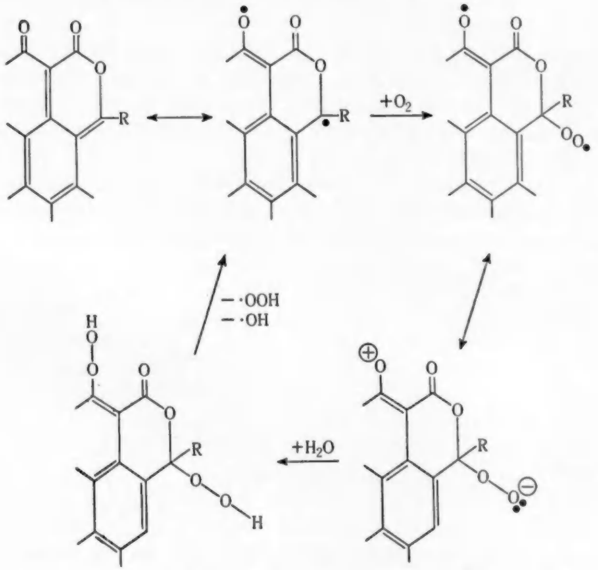


Fig. 6.—Proposed mechanism for photographic activity of carbon black (Russel effect).

IV. Experimental Procedure

Oxidation of Ferrous Thiocyanate : A Thunberg tube was charged with 25 ml of approx. 0·02N ferrous thiocyanate reagent (see Yule and Wilson 1931) and 0·010 g of carbon separately and the solution was frozen in liquid air. The tube was then evacuated and filled with pure argon and the operation repeated twice to remove traces of oxygen. After final evacuation the carbon was tipped, the solid was allowed to thaw, and the carbon was left in contact with the solution and shaken for 2 hr at room temperature. Blanks were run correspondingly. The colour was determined on a Spekker absorptiometer.

Extraction of H_2O_2 with Acid: Carbon (0·2 g) was shaken for 5 min at room temperature with 25 ml of 1N H_2SO_4 and centrifuged. To 15 ml of the extract, 3 ml of 0·005N $KMnO_4$ was added and the colour determined on the Spekker absorptiometer.

Photographic Exposure: Carbon samples were placed in small glass cavities of approx 1 c.c. volume and exposed in the dark for 72 hr at 70 °C to special Rapid Panchromatic plates with an air space of 2 mm between the samples and the photographic emulsion. The plates were then developed in the usual way.

Chromene Estimation: Carbon (1 g) was shaken at room temperature for 48 hr with 25 ml of 0·030N hydrochloric acid. Toluene (15 ml) was then added and the shaking continued for a further 96 hr. The mixture was then filtered through a sintered-glass filter, the phases were separated, and the aqueous layer titrated with standard alkali, using bromothymol blue as indicator.

Lead Tetra-acetate Test: Carbon (0·005 g) and glacial acetic acid (2 ml) were introduced into the centre compartment of a Warburg vessel containing lead tetra-acetate (0·3 g) in the side-arm. After equilibration at 30 °C overnight, the lead tetra-acetate was tipped into the centre compartment and the changes in pressure observed. In a separate run on a much larger scale, the evolved gas was analysed by gas chromatography after 2 hr.

Reaction with Hydroquinone: Carbon (1 g) was suspended in 0·4 per cent. aqueous hydroquinone (25 ml) for 24 hr. It was then filtered, thoroughly washed with water, and dried at 70 °C.

Reaction with Monomers: Small samples of carbon were exposed to monomer vapours in a closed system containing a reservoir of liquid monomer at 25 and 70 °C respectively for various times. The carbon was then washed with a suitable solvent and vacuum dried. Blanks were run correspondingly.

V. References

Barton, B. C., Smallwood, H. M., and Ganzhorn, G. H. (1954).—*J. Polym. Sci.* **113**: 487–98.

Bonhoeffer, K. F., Farkas, A., and Rummel, K. W. (1933).—*Z. phys. Chem.* B **21**: 225–34.

Burstein, R., and Frumkin, A. (1941).—*C.R. Acad. Sci. URSS* **32**: 327–9.

Criegee, R., Pilz, H., and Flygare, H. (1939).—*Ber. dtsch. chem. Ges.* B **72**: 1799–804.

Egerton, J. S. (1949).—*J. Soc. Dy. Col. Bradford* **65**: 764. (Proc. Soc. Dy. Col. Symp. on Photochemistry in relation to textiles.)

Garten, V. A., and Weiss, D. E. (1955).—*Aust. J. Chem.* **8**: 68–95.

Garten, V. A., and Weiss, D. E. (1957).—*Aust. J. Chem.* **10**: 309–28.

Garten, V. A., and Weiss, D. E. (1959).—*Industr. Chem.* (in press).

Garten, V. A., Weiss, D. E., and Willis, J. B. (1957).—*Aust. J. Chem.* **10**: 295–308.

Hallum, J. V., and Drushel, H. V. (1958).—*J. Phys. Chem.* **62**: 110–16.

Haslam, J. S. (1928).—*Fuel Lond.* **7**: 253–7.

Hock, H., and Lang, Sh. (1942).—*Ber. dtsch. chem. Ges.* **75**: 1051–4.

Jones, R. E., and Townend, D. T. A. (1950).—*J. chim. Phys.* **47**: 348–52.

Kautsky, H. (1931).—*Ber. dtsch. chem. Ges.* **64**: 2677.

Moureu, Ch., Dufraisse, Ch., and Dean, P. M. (1926*a*).—*C.R. Acad. Sci. Paris* **182**: 1440.

Moureu, Ch., Dufraisse, Ch., and Dean, P. M. (1926*b*).—*C.R. Acad. Sci. Paris* **182**: 1584.

Mukherjee, P. N., and Lahiri, A. (1957).—*Fuel Lond.* **36**: 176–81.

Rummel, K. W. (1933).—*Z. phys. Chem.* A **167**: 221–39.

Russel, W. J. (1908).—*Proc. Roy. Soc.* B **78**: 385.

Schenck, G. O. (1948).—*Naturwissenschaften* **35**: 28.

SCHENCK, G. O., and ZIEGLER, K. (1945).—*Naturwissenschaften* **32**: 157.
SCHENCK, G. O., and ZIEGLER, K. (1951).—*Naturwissenschaften* **38**: 356.
STUDEBAKER, M. L., HUFFMAN, E. W. D., WOLFE, A. C., and NABORS, L. G. (1956).—*Industr. Engng. Chem.* **48**: 162–6.
TREIBS, W. (1944).—*Ber. dtsch. chem. Ges.* **77**: 69–71.
TURKEVICH, J., and LAROCHE, J. (1958).—*Z. phys. Chem.* **15**: 399–408.
WATERS, W. A. (1950).—Inst. intern. chim. solvay, 8 Cons. Chim. Univ. Bruxelles. pp. **63–112**.
WINDAUS, A., and BRUNKEN, J. (1928).—*Liebigs Ann.* **460**: 225–35.
WINSLOW, N. M. (1947).—*Trans. Electrochem. Soc.* **92**: 411–25.
WITTIG, G., and LANGE, W. (1938).—*Ann. Chem.* **536**: 266–84.
YOHE, G. R., and HARMAN, C. A. (1941).—*J. Amer. Chem. Soc.* **63**: 555–6.
YULE, J. A. C., and WILSON, C. P. (1931).—*Industr. Engng. Chem.* **23**: 1254–9.
ZIEGLER, K., and EWALD, L. (1933).—*Liebigs Ann.* **504**: 162–81.

LIQUID–VAPOUR EQUILIBRIA

IX. THE SYSTEMS *n*-PROPANOL+BENZENE AND *n*-BUTANOL+BENZENE AT 45 °C

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Summary

Liquid-vapour equilibrium data are given for the systems *n*-propanol+benzene and *n*-butanol+benzene at 45·00 °C. The data are used to calculate the excess free energy of mixing for these systems.

I. Introduction

Liquid-vapour equilibria for the system *n*-propanol+benzene have been determined at 20 °C by Tryhorn and Wyatt (1928), at 40·0 °C by Lee (1931), and at 10 degree intervals from 0 to 70 °C by Schmidt (1926). Measurements have also been made at atmospheric pressure by Wehe and Coates (1955) and Bonauguri, Bicelli, and Spiller (1951) and at elevated pressures by Kumarkrishna Rao, Swami, and Narasinga Rao (1957).

The liquid-vapour equilibria for the system *n*-butanol+benzene have been determined at 20 °C by Tryhorn and Wyatt (1928) and at 25 °C by Allen, Lingo, and Felsing (1939). Measurements have also been made at atmospheric pressure by Bonauguri, Bicelli, and Spiller (1951) and by Shemilt (personal communication).

The present measurements were made to obtain values of the excess free energy of mixing at 45 °C as no published data of sufficient accuracy were available at this temperature. The data obtained here will be discussed in a subsequent paper together with those obtained previously for other alcohol systems.

II. Experimental

(*a*) *Apparatus*

The liquid-vapour equilibrium and vapour pressure measurements were made using the equilibrium still described by Brown (1952); pressure measurements were made by the methods of Brown and Ewald (1950) and the temperatures were measured with a calibrated Myer type platinum resistance thermometer and calibrated Mueller bridge using the methods described by Brown and Smith (1957). Liquid and vapour samples were analysed by refractive index measurements using a Hilger-Chance precision refractometer maintained at 25·00±0·01 °C.

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(b) *Purification and Properties of Components*

Benzene was purified by the method described by Brown and Ewald (1951). *n*-Propanol usually contains allyl alcohol as its major impurity and removal of this as the close-boiling azeotrope with *n*-propanol is wasteful if the allyl alcohol content is high even when an efficient distillation column is used. The method of Kretschmer (1951) gave a product with a high density and refractive index when the initial allyl alcohol content was above about 1 per cent. The method finally employed was to fractionally distil a sample containing less than 1 per cent. of allyl alcohol using the column described by Brown and Ewald (1951) operated at a reflux ratio of 75 : 1 and rejecting the head fractions with a high density. *n*-Butanol was purified by distillation using the same column.

TABLE 1

PHYSICAL PROPERTIES OF COMPONENTS

Property	Benzene	*n*-Propanol	*n*-Butanol
Boiling point/760 mm Hg (°C) . .	80·04	97·08	117·52
$d_4^{25\cdot00}$	0·87372	0·79958	0·80579
$n_D^{25\cdot00}$	1·49798	1·38312	1·39730
Vapour pressure 45·00 °C . .	223·64	69·35	25·34

The physical properties of the liquids used are shown in Table 1. The vapour pressures of the sample of benzene were checked at 45 °C and at the normal boiling point and found to be in good agreement with the values previously obtained by us. The vapour pressures of the alcohols were measured over a range of temperatures and the values found are given in Tables 2 and 3. These values were fitted by the method of Willingham *et al.* (1945) to the Antoine equation

$$\log_{10} P = A - \frac{B}{C+t},$$

where P is in standard mm Hg and t is in °C. The values of the constants found and σ, the standard deviation of the fit, are also presented in Tables 2 and 3.

TABLE 2

VAPOUR PRESSURE OF *n*-PROPANOL

t (°C)	P (mm Hg)	t (°C)	P (mm Hg)	Antoine Equation Constants
97·10	760·51	70·09	246·25	
97·09	760·05	65·80	201·52	A=7·83230
97·08	760·25	61·51	163·90	B=1485·84
94·36	685·12	54·52	115·55	C=203·00
91·13	603·59	48·18	82·64	σ=0·14 mm Hg
86·00	491·03	48·16	82·47	
81·11	400·38	48·14	82·43	
74·56	301·35			

TABLE 3

VAPOUR PRESSURE OF *n*-BUTANOL

t (°C)	P (mm Hg)	t (°C)	P (mm Hg)	Antoine Equation Constants
117·56	760·35	79·02	157·68	
113·26	651·47	79·00	157·38	$A=7\cdot56483$
108·23	540·33	75·39	132·70	$B=1416\cdot99$
103·30	446·65	70·15	102·52	$C=184\cdot99$
97·33	351·47	64·74	77·79	$\sigma=0\cdot21$ mm Hg
89·14	248·72	64·56	77·06	

(c) *Analytical Methods*

Mixtures were prepared from weighed amounts of the components and their compositions were calculated allowing for air buoyancy and for the weight of air-vapour mixture of the first component displaced on adding the second, their compositions and refractive indices are given in Tables 4 and 5. The method of Brown and Smith (1954) was employed to calculate the composition of the liquid and vapour samples from the refractive index measurements.

TABLE 4

n-PROPANOL + BENZENE : REFRACTIVE INDEX—COMPOSITION DATA

x_1	$n_D^{25\cdot00}$	x_1	$n_D^{25\cdot00}$
0·0530	1·49221	0·5063	1·44344
0·1080	1·48644	0·6146	1·43103
0·2041	1·47629	0·7075	1·42008
0·3150	1·46450	0·7928	1·40968
0·4180	1·45328	0·8914	1·39730
0·4998	1·44416	0·9492	1·38981

TABLE 5

n-BUTANOL + BENZENE : REFRACTIVE INDEX—COMPOSITION DATA

x_1	$n_D^{25\cdot00}$	x_1	$n_D^{25\cdot00}$
0·0478	1·49239	0·4973	1·44542
0·0987	1·48672	0·6060	1·43473
0·2007	1·47571	0·6960	1·42608
0·3096	1·46434	0·8093	1·41526
0·3986	1·45528	0·9034	1·40636
0·4881	1·44635	0·9546	1·40159

III. RESULTS

The liquid-vapour equilibrium data are given in Tables 6 and 7 where x_1 and y_1 are the mole fractions of the alcohol in the liquid and vapour respectively, P is the total pressure in standard mm Hg, and α is the relative volatility given by $\alpha=y_1x_2/y_2x_1$.

From graphs of the data shown in Table 6 it was concluded that the system n-propanol+benzene forms an azeotrope at 45·00 °C at a mole fraction of n-propanol of 0·132 and at a pressure of 239·0 mm Hg.

Graphs of the data given in Table 7 indicate that if an azeotrope is formed at 45 °C in the system n-butanol+benzene it is at a moie fraction of butanol less than 0·01.

TABLE 6

n-PROPANOL+BENZENE 45·00 °C : EXPERIMENTAL DATA AND DERIVED FUNCTIONS

x_1	y_1	P	α	μ_1^E	μ_2^E	G_x^E
0·0470	0·0843	235·26	1·867	1138·6	6·2	59·4
0·0977	0·1194	238·35	1·252	903·1	24·3	110·2
0·2144	0·1560	237·55	0·6770	572·1	83·1	187·9
0·2973	0·1728	234·89	0·4938	422·5	134·0	219·8
0·4061	0·1935	229·51	0·3509	282·0	210·0	239·2
0·4807	0·2080	224·13	0·2837	206·0	268·7	238·5
0·5252	0·2185	220·42	0·2528	170·5	307·5	235·5
0·6053	0·2402	211·04	0·2061	113·3	378·7	218·1
0·7033	0·2790	194·66	0·1632	62·2	475·8	184·9
0·7982	0·3437	170·06	0·1324	29·1	576·1	139·5
0·9140	0·5252	122·08	0·1041	3·6	704·1	63·8

TABLE 7

n-BUTANOL+BENZENE 45·00 °C : EXPERIMENTAL DATA AND DERIVED FUNCTIONS

x_1	y_1	P	α	μ_1^E	μ_2^E	G_x^E
0·0538	0·0323	221·29	0·5870	1042	7·8	63·4
0·0974	0·0432	218·90	0·4184	842·7	23·7	103·5
0·1986	0·0579	212·80	0·2480	559·0	71·6	168·4
0·3007	0·0684	205·68	0·1708	380·4	129·5	204·9
0·4004	0·0779	197·33	0·1265	255·6	194·4	218·9
0·4915	0·0885	187·62	0·1005	174·8	259·8	218·0
0·6003	0·1054	171·29	0·0784	101·7	343·4	198·3
0·7072	0·1311	148·84	0·0625	47·8	434·0	160·9
0·8206	0·1893	114·04	0·0511	18·6	533·1	110·9

IV. DERIVED THERMODYNAMIC FUNCTIONS

The excess chemical potentials μ_1^E and μ_2^E and the excess free energy of mixing G_x^E were calculated from the equilibrium data using equations (2), (3), and (4) of Brown and Smith (1954). The values obtained (cal/g-mol of mixture) are shown in Tables 6 and 7. The liquid molar volumes V_1 and V_2 required for these calculations were obtained from densities given by Timmermans (1950). The value of the second virial coefficient for benzene vapour at 45 °C was obtained from equation (A5) of Allen, Everett, and Penney (1952). The second virial coefficients for n-propanol and n-butanol were calculated by corresponding states from the experimental data of Kretschmer and Wiebe (1954) for ethanol and

*iso*propanol. The values of the critical data used for *n*-propanol were those of Kobe and Lynn (1953) and for *n*-butanol those of Singh and Shemilt (1955). An attempt was made to estimate the value of the second virial coefficient for *n*-butanol at 45 °C from the high temperature *P-V-T* data of Shemilt, Esplen, and Singh (1959) using the method of David and Hamann (1957). Values of $(Z-1)V^*$ were plotted against $1/V^*$ for values of T^* from 0·84 to 0·92, where $Z=PV/RT$, $V^*=V/V_c$, and $T^*=T/T_c$. The plots curved badly at low values of $1/V^*$ for T^* above about 0·9 and the data were not available at sufficiently low temperatures to enable a virial coefficient value at 45 °C ($T^*=0\cdot578$) to be obtained. The second virial coefficient, β_{12}, in the equation of state for the mixed vapours, and the allied coefficient δ_{12} were calculated by the method used by Brown and Smith (1954).

For the system *n*-propanol+benzene at 45·00 °C the values of the second virial coefficients and molar volumes (l/mol) were taken to be:

β_{11}	β_{22}	β_{12}	δ_{12}	V_1	V_2
−3·23	−1·23	−1·09	+2·29	0·08	0·092

For the system *n*-butanol+benzene the values used were:

β_{11}	β_{22}	β_{12}	δ_{12}	V_1	V_2
−4·88	−1·23	−1·18	+3·76	0·094	0·092.

V. Discussion

The excess free energy data were fitted by the method of least squares to equation (1)

$$G_x^E=x_1x_2[a+b(x_1-x_2)+c(x_1-x_2)^2]. \qquad (1)$$

For the system *n*-butanol+benzene at 45·00 °C the values of the constants (cal/g-mol) and the standard deviation of the fit (σ) were:

a	b	c	σ
+949·2	−224·2	+136·6	1·9 .

These values do not indicate separation into two liquid phases. The thermodynamic consistency of the data was tested by the method used previously, Brown and Smith (1954) and found to be satisfactory; the ratio of areas above and below datum on a plot of $\mu_1^E-\mu_2^E$ against x_1 was 1·03. Errors in G_x^E due to errors in the directly measured quantities were estimated to be ±3·5 cal/mol. Values of G_x^E at 40 °C were calculated from the data of Lee (1931), these were about 4 per cent. higher than the values given here. Values of G_x^E were also calculated from the less accurate data of Schmidt (1926) at 20 and at 40 °C using the method of Barker (1953). These data give values of G_x^E at $x_1=0\cdot5$ of 270 cal/mole at 20 °C and 280 cal/mole at 40 °C compared with the value of 237·3 at 45 °C found in this work.

For the system *n*-butanol+benzene at 45·00 °C the values of the constants obtained by fitting the excess free energy data to equation (1) were:

a	b	c	σ
+861·5	−250·3	+142·5	1·8.

In the test for thermodynamic consistency the ratio of the areas was 1·04 and the errors in G_x^E due to those in the measured quantities were estimated to be ± 3 cal/mole at $x_1=0\cdot 2$ and ± 7 cal/mole at $x_1=0\cdot 8$. Values of G_x^E calculated from the data of Allen, Lingo, and Felsing (1939) for this system at 25 °C are about 20 per cent. higher than those given here.

VI. References

Allen, B. B., Lingo, S. P., and Felsing, W. A. (1939).—*J. Phys. Chem.* **43**: 425.

Allen, P. W., Everett, D. H., and Penney, M. F. (1952).—*Proc. Roy. Soc.* A **212**: 149.

Barker, J. A. (1953).—*Aust. J. Chem.* **6**: 207.

Brown, I. (1952).—*Aust. J. Sci. Res.* A **5**: 530.

Brown, I., and Ewald, A. H. (1950).—*Aust. J. Sci. Res.* A **3**: 306.

Brown, I., and Ewald, A. H. (1951).—*Aust. J. Sci. Res.* A **4**: 198.

Brown, I., and Smith, F. (1954).—*Aust. J. Chem.* **7**: 264.

Brown, I., and Smith, F. (1957).—*Aust. J. Chem.* **10**: 423.

Bonauguri, E., Bicelli, L., and Spiller, G. (1951).—*Chem. e Ind.* **33**: 81.

David, H. G., and Hamann, S. D. (1957).—Proc. of I.U.P.A.C. Conference on thermodynamic and transport properties of fluids. p. 74.

Kobe, K. A., and Lynn, R. E. (1953).—*Chem. Rev.* **52**: 117.

Kretschmer, C. B. (1951).—*J. Phys. Chem.* **55**: 1351.

Kretschmer, C. B., and Wiebe, R. (1954).—*J. Amer. Chem. Soc.* **76**: 2579.

Kumarkrishna Rao, V. N., Swami, D. R., and Narasinga Rao, M. (1957).—*J. Sci. Indian Res.* B **16**: 23.

Lee, S. C. (1931).—*J. Phys. Chem.* **35**: 3558.

Schmidt, G. C. (1926).—*Z. phys. Chem.* **121**: 221.

Shemilt, L. W., Esplen, R. W., and Singh, R. (1959).—*Canad. J. Chem. Engng.* (in press).

Singh, R., and Shemilt, L. W. (1955).—*J. Chem. Phys.* **23**: 1370.

Timmermans, J. (1950).—" Physico-Chemical Constants of Pure Organic Compounds." (Elsevier Book Co.: New York.)

Tryhorn, F. G., and Wyatt, W. E. (1928).—*Trans. Faraday Soc.* **24**: 40.

Wehe, A. H., and Coates, J. (1955).—*Amer. Inst. Chem. Eng. J.* **1**: 241.

Willingham, C. B., Taylor, W. J., Pignocco, J. M., and Rossini, F. D. (1945).—*J. Res. Nat. Bur. Stand.* **35**: 219.

TWO-LAYER ADSORPTION OF DODECYL SULPHATE ON BARIUM SULPHATE

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Summary

The adsorption of dodecyl sulphate on barium sulphate has been measured as a function of concentration. Comparison with krypton adsorption and stearic acid adsorption has shown that an amount of dodecyl sulphate equivalent to two layers was adsorbed at saturation, in agreement with some, but not all, previous reports for such systems.

The possibility that precipitation of barium dodecyl sulphate may have contributed to the apparent adsorption has been specially investigated and the effect was shown to be small. Indirect evidence in support of two-layer adsorption has also been found from sedimentation studies. The adsorption of a second layer, with hydrophilic groups oriented away from the solid, provides the most satisfactory explanation of rewetting at high concentrations of adsorbate.

I. Introduction

There is an extensive literature concerning the adsorption of surface active compounds from aqueous solution on solids. However, very few workers have made quantitative adsorption measurements. Much of the work has involved practical systems, especially those occurring in the flotation of minerals, and in these, initial impurities, side reactions, and difficulties of assay have made quantitative, or even reliable qualitative work impossible. As a result, little is known with certainty about the relationship between wetting properties and surface coverage, or about the maximum amount of adsorbate which can be taken up by a surface.

The adsorption of amphipathic ions on a metal or mineral surface from aqueous solution causes it, at low adsorbate concentrations, to become hydrophobic. At higher concentrations the surface may resume its hydrophilic character. It is generally agreed that the hydrophobic behaviour results from the adsorption of ions with their non-polar chains oriented outwards, presenting a paraffin-like surface to the water. Agreement has not been reached, however, on the mechanism of the rewetting phenomenon. Two theories have been proposed which are worth considering.

(i) Held and Samochwalov (1935) and Held and Khainsky (1936) suggested that a second layer of long-chain ions is adsorbed, in which the hydrophilic polar groups are oriented away from the surface.

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(ii) Wark (1936) suggested that the contact between a bubble of air and a mineral surface might be hindered by an " armouring " of the air/water interface by adsorbed amphipathic ions.

Experiments indicating two-layer adsorption have been reported by several workers. Held and co-workers compared the saturation adsorption of soaps from aqueous solution with the saturation adsorption of the corresponding fatty acids from benzene solution. They found the soap adsorption to be twice the acid adsorption on both cinnabar (HgS) and barium sulphate. Schulman and Smith (1952) obtained a similar result for adsorption on copper.

In these cases there was no indication from the adsorption isotherm of the two-step nature of the adsorption. However, Cuming and Schulman (1953) have shown that the adsorption isotherms for dodecyl sulphate on copper contained two steps of equal height, and that the copper was hydrophobic at concentrations corresponding to the first step and hydrophilic at concentrations corresponding to the second step. Four different methods, which were used to determine the specific area of the powder, were all consistent with the interpretation that the first step corresponded to a monolayer and the second step to two layers.

However, contrary to all of these results, Edwards and Ewers (1951) found the saturation adsorption of cetyl sulphate on cassiterite (SnO_2) to be equivalent to a monolayer only, when compared with the specific area measured by low temperature nitrogen adsorption and with the estimated specific geometric area.

It has been shown by Wark and others that the maximum contact angle obtained by the adsorption of amphipathic substances is independent, within experimental error, of the nature of the adsorbent (Sutherland and Wark 1955). It follows that the surfaces of two different solids, when covered by identical monolayers, are very similar, and it is therefore unlikely that in one case a second layer of amphipathic ions is adsorbed, and in another it is not : that on $BaSO_4$ or copper there can be two layers at saturation and on SnO_2 only one. If a monolayer is the maximum level of adsorption attainable in each case, then in the experiments of Held and co-workers, Schulman and Smith, and Cuming and Schulman, one-half of the uptake measured must have occurred by processes other than adsorption ; the anionic adsorbates they used may, for example, have been precipitated from solution by polyvalent cations dissolved from the solid surfaces. This possibility will be discussed in more detail below.

If, on the other hand, a double layer can be formed, the adsorption of cetyl sulphate measured by Edwards and Ewers represented a coverage by two layers of only one-half of the surface available to nitrogen molecules. These authors claimed that their SnO_2 powder was non-porous, on the evidence of electron micrographs from which they estimated the average particle size. In fact, however, the electron micrograph in their paper indicated some degree of sintering, and it is possible that pores could have been present which would admit nitrogen but not cetyl sulphate ions. A further possibility also exists, namely, that only a fraction (in this case one-half) of the solid surface was reactive in chemisorption. Either of these circumstances—or a combination of both—could bring about the appearance of monolayer saturation when a double layer is in fact adsorbed.

Further evidence has therefore been sought in a study of the adsorption of dodecyl sulphate on barium sulphate, in which particular attention has been paid to the possibility that precipitation of the adsorbate might be mistaken for adsorption. As well as direct measurements of the dodecyl sulphate removed from solution by barium sulphate, some sedimentation results are included which provide further indirect evidence of two-layer adsorption.

II. Experimental

(a) *Materials*

The sodium dodecyl sulphate (kindly supplied by Dr. E. D. Goddard of Lever Bros. Ltd.) had been found to give no dip in the surface tension-concentration curve at the c.m.c. (Goddard, Harva, and Jones 1953). The stearic acid (m.p. $=69 \cdot 1$–$69 \cdot 3$ °C) was a sample purified in this laboratory. Inorganic reagents were of A.R. quality and organic solvents were redistilled. Laboratory distilled water was either passed through a column of "Biodeminrolit FF" resin, or redistilled in the presence of alkaline $KMnO_4$. Krypton (supplied by the British Oxygen Co.) was purified by distillation in the vacuum apparatus; helium (supplied by the Mond Laboratory, Cambridge) was dried and passed over charcoal at 78 °K.

Barium sulphate was prepared in several batches by precipitation from H_2SO_4 and $BaCl_2$ by the very slow addition (3–4 ml/min) simultaneously of 500 ml of $0 \cdot 5$M solutions of the reagents to $2 \cdot 5$ l. of N HCl solution at 90 °C with vigorous stirring. The temperature was maintained and stirring was continued for several hours in order that the growth of larger crystals should continue at the expense of the smaller ones. The product was washed with several litres of twice distilled water, and in this process very fine particles were removed by decantation. Finally, the powder was heated in air for 30 min at 400–500 °C and the batches mixed together. Most of the particles were well-formed crystals with an average size of about 5–10 μ (maximum about 20 μ), though there were a few aggregates which included smaller particles.

(b) *Krypton Adsorption*

Standard techniques were employed to determine adsorption isotherms for krypton at 78 °K. Pressures and volumes were both measured with a two-stage McLeod gauge. Corrections for gas imperfections and for thermomolecular flow (Kington and Holmes 1953) were negligible in the pressure range used (0–3 mm Hg). The reproducibility was about ± 2 per cent.

The results were treated according to the B.E.T. method (Brunauer, Emmett, and Teller 1938). The vapour pressure of liquid krypton, extrapolated to the adsorption temperature, was used in the B.E.T. equation, in preference to the solid vapour pressure actually measured (Beebe, Beckwith, and Honig 1945).

The molecular area of adsorbed krypton is uncertain. Values ranging from $14 \cdot 6$ to 21 Å^2 have been suggested but empirical comparisons of the adsorption of krypton with that of other gases on a number of solids seem to indicate that a

value high in this range should be used (Beebe, Honig, and Beckwith 1945; Davis, de Witt, and Emmett 1947; Tiley 1950). A molecular area of 20 Å^2 has therefore been assumed in the present work.

(c) *Stearic Acid Adsorption*

Weighed samples of powder were shaken in glass-stoppered tubes with 3 to 10 ml of a solution of stearic acid in light petroleum (b.p. 80–100 °C), and samples were removed at intervals. The concentrations of the initial solution and of the samples were determined by spreading them on dilute HCl in a Langmuir trough. Adsorption was complete in a few minutes. Measurements were made at a series of concentrations and the specific area was calculated from the adsorption at saturation. It was assumed that the monolayer formed at saturation was close-packed, with a molecular area of 20 Å^2 (Harkins and Gans 1931; Smith and Fuzek 1946).

Lancaster (1953) has investigated the effects of traces of water, both on the solid surfaces and in solution, on the adsorption of fatty acids on powders. On unreactive solids water was found to have a blocking effect whilst on reactive solids it promoted corrosion. In the present work, therefore, the powder was carefully dried by evacuation at temperatures between 50 and 150 °C and broken directly into the solution, the latter being prepared from light petroleum dried over sodium.

(d) *Sodium Dodecyl Sulphate Adsorption*

Weighed samples of powder were shaken with NaDS solutions of various concentrations, which were analysed before and after adsorption and the amounts adsorbed determined by difference. Shaking was done mechanically in a thermostat at 25 °C. It was necessary to centrifuge briefly before removing aliquots of the solution.

The NaDS solutions were analysed by titration against cetyl trimethylammonium bromide to the equipartition of methylene blue between the aqueous phase and chloroform. It was found unnecessary to add Na_2SO_4 and H_2SO_4 as recommended by Epton (1948). The titrations were reproducible to better than 1 per cent. The weight-volume ratio in the adsorption systems was always such that the concentration was reduced by at least 25 per cent. Thus the accuracy of the measurements was better than ± 4 per cent.

III. Results

(a) *Monolayer Adsorption Capacity*

The monolayer adsorption capacities for the krypton and stearic acid were found to be $8\cdot5\times10^{-7}$ and $9\cdot5\times10^{-7}$ mole g^{-1} respectively. Both of these adsorbates will be assumed (see above) to have a molecular area of 20 Å^2. The area occupied by the DS$^-$ ion on a solid surface is not known from direct measurements. However, there is evidence from measurements on adsorbed and spread films of long-chain sulphates at the air–water interface (Pethica and Few 1952; Matijevic and Pethica 1958) that under conditions where the charge on the $-SO_4^-$ group is effectively neutralized the limiting area per ion is about

20 Å^2. In fact the spacing of Ba^{++} ions in the $BaSO_4$ surface is probably an important factor, but in the absence of data on the nature and relative areas of the crystal faces exposed, it will be assumed that the ions are close-packed and the limiting value will be used. Taking the mean of the stearic acid and krypton results, the monolayer capacity of the powder for dodecyl sulphate was then 9×10^{-7} mole g^{-1}. Further evidence which supports the accuracy of this estimate will be given below (Subsection (*b*) (iii)).

(*b*) *Adsorption of Dodecyl Sulphate*

(i) *Adsorption on Separate Samples.*—When the adsorption isotherm was determined in the conventional way, by determining the adsorption on a different sample of powder for each concentration of solution used, the full curve in Figure 1 was obtained. It is seen that the saturation level is 22 mole g^{-1}, which is equivalent to rather more than two monolayers.

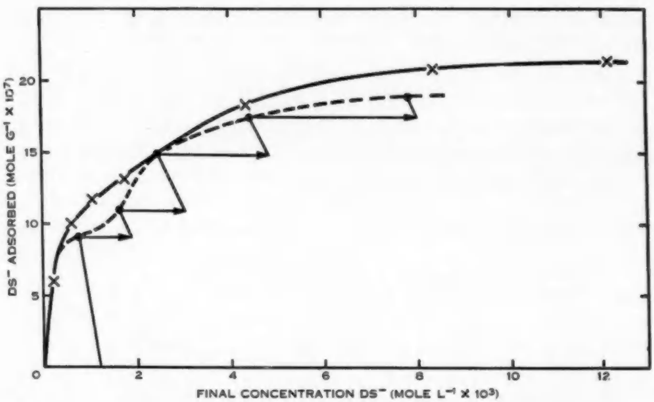


Fig. 1.—Adsorption of dodecyl sulphate on $BaSO_4$, concentration isotherms.
—— Adsorption on separate samples from solutions of different concentration (30 min).
-- Adsorption in stages on one sample by successive increases of concentration (10 min between stages).
—→ Nominal concentration increases.

The result is similar to that obtained by Held and Samochwalov (1935) and Held and Khainsky (1936) for soaps on barium sulphate. However, their conclusions have been criticized because the same apparent absorption could have resulted from bulk precipitation of barium soap by Ba^{++} ions released into solution from the solid, so that their curves represented the sum of monolayer adsorption and precipitation. Such a criticism is equally applicable to the present results. Further experiments have therefore been carried out in order to assess the contribution, if any, of bulk precipitation of $Ba(DS)_2$.

The saturation concentration of Ba^{++} ion (10^{-5}M) corresponds, in the volume of solution used, to a negligible proportion of the amount of DS^- removed from

the solution at saturation. There are, however, three possible processes to consider:

(1) Ba^{++} ions may dissolve from the surface and precipitate as $Ba(DS)_2$ at a rate comparable with the rate of adsorption;

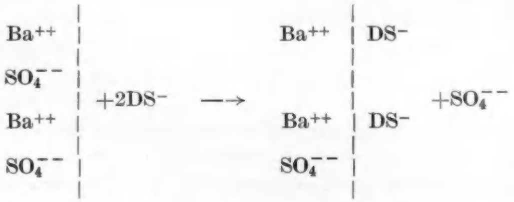
(2) Ba^{++} ions may dissolve after adsorption is complete, that is, they may penetrate the adsorbed film of DS^- ions;

(3) $Ba(DS)_2$ may be detached from the solid surface and aggregate in the solution, allowing further adsorption to take place.

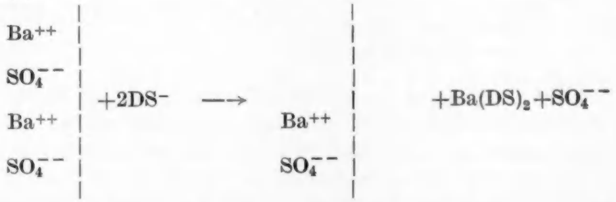
Mechanisms (2) and (3) were investigated by following the disappearance of DS^- with time at various concentrations. Reaction was substantially complete after 5 or 10 min, but there followed a slow uptake of DS^-, linear with time, amounting to *c.* 15 per cent. of the initial uptake per hour. Evidently one of these mechanisms operates, or perhaps both, but it is clear that the process is slow and cannot account for the reaction of dodecyl sulphate equivalent to a second monolayer within the 30 min allowed for adsorption in determining the isotherm.

(ii) *Adsorption in Stages.*—The only possibility of appreciable precipitation then is by mechanism (1). In order to evaluate its importance, the adsorption isotherm was determined in a different way. In the experiments with separate samples, at each concentration of DS^- ion a clean surface of $BaSO_4$ was available for the competing reactions (*a*) and (*b*):

Reaction (*a*)



Reaction (*b*)



The solubility product of $Ba(DS)_2$ was determined using the CTAB titration method and found to be *c.* 10^{-12} mole^3 l^{-3}. It can be readily shown from this that the concentration of DS^- corresponding to the first point plotted on the full curve in Figure 1 is too low for the precipitation of $Ba(DS)_2$ from a saturated solution of $BaSO_4$ (10^{-5}M); even at the concentration represented by the second point

practically all of the DS^- which is removed from solution must be adsorbed rather than precipitated.

In the new experiment, adsorption was allowed to take place in stages on one sample of $BaSO_4$ by adding successive, exact amounts of NaDS, and after each addition, shaking for 10 min and determining the concentration. A typical isotherm is given as the broken curve in Figure 1. Changes in the steps taken caused only minor variations in shape but no change in the saturation level. After the first step the solid surface was covered—or almost covered—with a monolayer. Thus at the higher concentrations attained in the subsequent stages, when the solubility product was in fact exceeded, the precipitation reaction (*b*) could occur only by the release of Ba^{++} ions through the closely packed hydrocarbon chains. The rate experiments have shown this to be a slow process, and if the uptake of DS^- ion above the monolayer level in the original experiment had been due entirely to precipitation, the isotherm obtained by adsorption in stages would not be expected to rise appreciably after the first stage. In fact the saturation value was now quite closely equivalent to two adsorbed layers. The fact that it was less than the previous value shows that a small amount of precipitation must have occurred in the single-stage experiments.

It is interesting to notice the appearance of a point of inflexion in the isotherm when the adsorption occurs in stages. This could be the result of a lower rate of adsorption at the commencement of the second layer, the 10 min interval being insufficient for complete adsorption at this concentration. Whatever its exact explanation, the kink in the isotherm can probably be regarded as an indication of the monolayer level of adsorption.

(iii) *The Effect of Ba^{++} Ion*.—Confirmation that the monolayer capacity was close to 9×10^{-7} mole g^{-1} emerged from the adsorption isotherm for a sample of powder whose surface was contaminated by soluble Ba^{++} ions arising from surface reduction during ignition (Fig. 2). The presence of the positive potential-determining ions caused strong monolayer adsorption at low concentrations, and adsorption was complete at *c*. 10^{-4}M, resulting in a distinct plateau which is seen more clearly in the inset. In this case the formation of a second adsorbed layer was obscured or prevented by the precipitation of $Ba(DS)_2$ which occurred at concentrations greater than *c*. 2×10^{-4}M. This minimum concentration for precipitation is sharply defined, and the shape of the curve is quite unlike the isotherms for the clean powder.

It is interesting to record that very similar curves have been obtained for freshly ground chalcopyrite, which is inevitably contaminated by soluble products of surface oxidation. This underlines the need for extreme caution in interpreting adsorption experiments with sulphide minerals.

(*c*) *Sedimentation Behaviour*

O'Connor and Buchanan (1953) have shown that the adsorption of alkyl sulphate ions on a positively charged solid, such as $BaSO_4$ in water (Buchanan and Heymann 1949), lowers the positive potential and at quite a low coverage makes it negative. In the present work it has been found that the sedimentation

rate (which is an indication of potential) is so sensitive to changes in the electrolyte concentration of the solution as to be explicable only by assuming that there is a layer of dodecyl sulphate ions oriented with their charged groups towards the solution.

The effect of electrolytes on an adsorbed film oriented in this way can be predicted from the behaviour of films of alkyl sulphates at air/water and oil/water interfaces. Pethica and Few (1954) have recently investigated these systems, and have found marked changes in the force area curves and in the surface-potentials of spread films of sodium octadecyl sulphate at a critical NaCl concentration of 0·3M. Conforming with this, they found that foams and paraffin emulsions, stabilized by NaDS, became unstable at the same NaCl concentration. They ascribed the effects to discharge of the $-SO_4^-$ groups by the Na^+ ions.

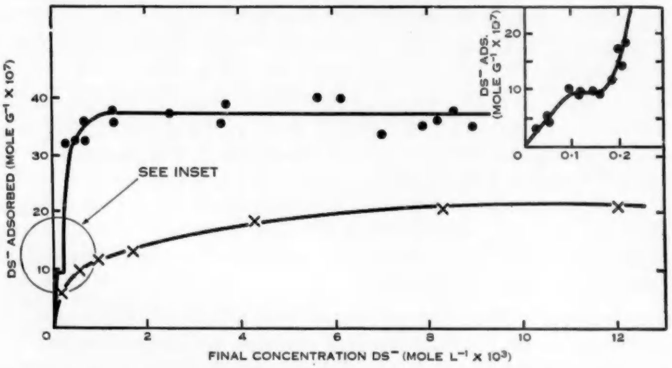


Fig. 2.—Effect of Ba^{++} ion contamination on the adsorption of dodecyl sulphate on $BaSO_4$.

● Contaminated powder.

× Powder washed free of Ba^{++}.

Barium sulphate suspensions are extremely stable in NaDS solutions due to the high negative potential imparted by the adsorbed film. Addition of NaCl, however, produced precisely the same effect as upon the other dispersed systems, namely, a sharp decrease in stability at a concentration of 0·3M. To a series of graduated tubes containing identical amounts of $BaSO_4$ suspended in 10 ml of 10^{-2}M NaDS, were added varying amounts of NaCl. After shaking, the tubes were allowed to stand, and the volume of sediment measured as a function of time. The curve in Figure 3, which shows the volume to which the solid had settled after 2½ hr, at various NaCl concentrations, is practically identical with the curve obtained by Pethica and Few for foam stabilities.

Such a parallel sensitivity to electrolyte concentration would be most unlikely if in the one case charged sulphate groups, and in the other case hydrocarbon chains, were directed towards the aqueous phase. In foams and emulsions the sulphate groups are oriented towards the water, and it follows that on the

solid surface there must be a similar layer of long-chain ions. Since the sulphate groups of the chemisorbed monolayer are attached to the surface, there must be a second amphipathically adsorbed layer with the reverse orientation.

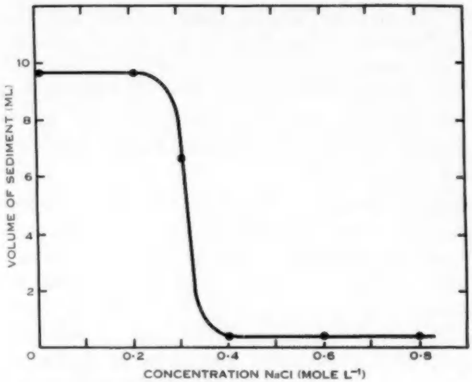


Fig. 3.—Effect of NaCl on the sedimentation of $BaSO_4$ in 10^{-2}M NaDS solution. Volume of sediment after 2½ hr.

IV. Discussion

Since it has been established in the present work that the experimental adsorption isotherm represented true adsorption, including little if any precipitation of adsorbate, and that the adsorption proceeded to two layers at saturation, it can be safely concluded that the two-layer isotherms previously reported for other systems also represented true adsorption.

The adsorption of two layers at saturation is the behaviour to be expected from the known properties of amphipathic ions in solution. At high concentrations they aggregate into micelles as a result of the tendency of their hydrocarbon chains to escape the aqueous environment; the ions are clustered with their chains in contact and their ionized groups facing outwards towards the water. It is therefore reasonable to expect that when a monolayer is *chemisorbed* on a solid, there will be *amphipathic adsorption* of a second layer of ions, with their polar groups oriented towards the aqueous phase. The double layer which results is equivalent to a flat micelle attached to the solid surface. Since the process is adsorption, rather than aggregation, it will not necessarily occur as suddenly with respect to concentration as micelle formation, but it might be expected to be complete at a concentration somewhere near the critical micelle concentration (c.m.c.). This is found to be the case, both in the present experiments and in previous work (Held and Samochwalov 1935; Held and Khainsky 1936; Schulman and Smith 1952; Cuming and Schulman 1953).

The adsorption of a second layer of ions with their ionized groups oriented away from the solid provides a reasonable explanation of rewetting at high concentrations, and comparison of the present results with wetting behaviour

strengthens this view. A direct comparison of rewetting and adsorption can be made using the present results and the contact angle-concentration data of Schulman and Leja (1954) for the NaDS–$BaSO_4$ system. The comparison shows that the contact angle starts to fall as adsorption increases above the monolayer level; it becomes zero at 6×10^{-3}M, at which concentration the second layer is almost complete. Further, it has been noted for a variety of systems that rewetting occurs at about the c.m.c. (Sutherland and Wark 1955; O'Connor and Sanders 1956). The several observations, including the present one, that two-layer adsorption is complete near the c.m.c., suggest strongly that the two phenomena are related, and that for any one system a fairly exact correlation will be found as in the present case.

The "armouring" theory of Wark (1936) implies that changes at the air/water interface are alone responsible for rewetting, and that two-layer adsorption need not be assumed. Indeed, the decrease in the free energy, γ_{LV}, of the air/water interface with increase of concentration will tend to favour the condition for rewetting, that $\gamma_{SV} - \gamma_{SL} > \gamma_{LV}$, where γ_{SV} and γ_{SL} are the free energies of the solid-air and solid-water interfaces. O'Connor and Sanders have shown in fact that when the maximum contact angle reached in the hydrophobic range is small, a change in γ_{LV} alone may be sufficient to account for rewetting. Their analysis shows, however, that in many cases $\gamma_{SV} - \gamma_{SL}$ must pass through a minimum. They point out that the increase in $\gamma_{SV} - \gamma_{SL}$ beyond this minimum can most reasonably be accounted for by assuming *additional adsorption* within the liquid phase at the solid-liquid interface, which would result in a further decrease in γ_{SL}. When the contact angle data of Schulman and Leja are examined in these terms it is readily seen that the dodecyl sulphate–barium sulphate system is of this type. It is clear therefore that in such systems a decrease in γ_{LV} is not a sufficient condition for rewetting, but that further adsorption must occur at the solid-liquid interface. The present results are consistent with this conclusion.

V. Acknowledgments

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VI. References

Beebe, R. A., Beckwith, J. B., and Honig, J. M. (1945).—*J. Amer. Chem. Soc.* **67**: 1554.
Brunauer, S., Emmett, P. H., and Teller, E. (1938).—*J. Amer. Chem. Soc.* **60**: 309.
Buchanan, A. S., and Heymann, E. (1949).—*J. Colloid Sci.* **4**: 137.
Cuming, B. D., and Schulman, J. H. (1953).—"Recent Developments in Mineral Dressing." p. 5. (Institution of Mining and Metallurgy: London.)
Davis, R. T., de Witt, T. W., and Emmett, P. H. (1947).—*J. Phys. Chem.* **51**: 1232.
Edwards, G. R., and Ewers, W. E. (1951).—*Aust. J. Sci. Res.* A **4**: 627.
Epton, S. R. (1948).—*Trans. Faraday Soc.* **44**: 226.
Goddard, E. D., Harva, O., and Jones, T. G. (1953).—*Trans. Faraday Soc.* **49**: 980.
Harkins, W. D., and Gans, D. M. (1931).—*J. Amer. Chem. Soc.* **53**: 2804.
Held, N. A., and Khainsky, I. A. (1936).—*Kolloidzschr.* **76**: 26.
Held, N. A., and Samochwalov, K. (1935).—*Kolloidzschr.* **72**: 13.
Kington, G. L., and Holmes, J. M. (1953).—*Trans. Faraday Soc.* **49**: 425.

Lancaster, J. K. (1953).—*Trans. Faraday Soc.* **49**: 1090.
Matijevic, E., and Pethica, B. A. (1958).—*Trans. Faraday Soc.* **54**: 1382.
O'Connor, D. J., and Buchanan, A. S. (1953).—*Aust. J. Chem.* **6**: 278.
O'Connor, D. J., and Sanders, J. V. (1956).—*J. Colloid Sci.* **11**: 158.
Pethica, B. A., and Few, A. V. (1954).—*Disc. Faraday Soc.* **18**: 258.
Schulman, J. H., and Leja, J. (1954).—*Trans. Faraday Soc.* **50**: 598.
Schulman, J. H., and Smith, T. D. (1952).—*Kolloidzschr.* **126**: 20.
Smith, H. A., and Fuzek, J. F. (1946).—*J. Amer. Chem. Soc.* **68**: 229.
Sutherland, K. L., and Wark, I. W. (1955).—"Principles of Flotation." (Australian Institute of Mining and Metallurgy: Melbourne.)
Tiley, P. F. (1950).—*Disc. Faraday Soc.* **8**: 201, 202.
Wark, I. W. (1936).—*J. Phys. Chem.* **40**: 661.

NEW OBSERVATIONS ON THE LOW TEMPERATURE REACTION BETWEEN HYDROGEN SULPHIDE AND SULPHUR DIOXIDE: A NOVEL METHOD FOR ANALYSING MILLIGRAM QUANTITIES OF HYDROGEN SULPHIDE IN THE PRESENCE OF CARBON DIOXIDE

By I. K. GREGOR* and R. L. MARTIN†

[*Manuscript received December 19, 1958*]

Summary

When an anhydrous mixture of liquid hydrogen sulphide and liquid sulphur dioxide contained in a sealed tube at —78 °C is gently warmed, the liquid remains clear until the temperature reaches *c.* —40 °C, when sulphur is slowly deposited. Careful measurements reveal that the two compounds always react in the mole ratio, $H_2S : SO_2 = 3 : 2$, which contrasts with the stoichiometric equation $2H_2S + SO_2 = 2H_2O + 3S$, widely quoted for their reaction in the moist gaseous phase or in aqueous solution.

This low temperature reaction forms the basis of a novel high vacuum method for estimating milligram quantities of hydrogen sulphide when in the presence of gases, such as carbon dioxide, whose volatilities are sufficiently similar to preclude the use of normal low-temperature vacuum fractionation methods.

I. INTRODUCTION

Innumerable references are contained in the literature concerning the reaction between hydrogen sulphide and sulphur dioxide at room temperature, both in aqueous solution and in the moist gaseous state, and although it is generally agreed that sulphur is a product of the reaction, usually formulated as:

$$2H_2S + SO_2 = 2H_2O + 3S,$$

it does not appear that a rigorous study of the products and of the stoichiometry of the reaction has been carried out. In contrast, very few investigations have been made of the reaction between these two substances at lower temperatures when both reactants are initially in the liquid state. One of the first reports appears to be that of Lang and Carson (1905), who observed that liquid hydrogen sulphide and liquid sulphur dioxide reacted slowly over a period of days with the deposition of sulphur and the formation of water. No temperature was specified and it was not made clear whether the reaction took place in a closed system. In the same year, Antony and Magri (1905) commented that the two liquids require the presence of an ionizing liquid to induce a reaction between them, perhaps a somewhat surprising conclusion when it is considered that both liquid hydrogen sulphide and liquid sulphur dioxide are well-established ionizing media. Subsequently, Quam (1925) contradicted their work by claiming that dry sulphur

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dioxide reacts vigorously with liquid hydrogen sulphide, but he gave no indication of the physical state of the sulphur dioxide or of the nature of the reaction products, or of the stoichiometry of the reaction. A year later, Mathews (1926) stated that a liquid hydrogen sulphide and liquid sulphur dioxide mixture, in the proportion 2 : 1, remained quite clear even after a period of 2 hr; again no experimental details or description of the reaction conditions were given. He further commented that no residue remained in the tube when the liquids evaporated, except at the exit tube where a deposit of sulphur was formed. This was ascribed to the influence of water which had condensed at the exit.

In the present paper, some new observations are described which provide the first quantitative data on the mole ratio in which the two compounds interact. These were made under strictly anhydrous conditions, during the course of a wider investigation by Gregor and Martin (1958, unpublished data) into the reaction between hydrogen sulphide and ozonized oxygen.

II. The Reaction between Hydrogen Sulphide and Sulphur Dioxide at Low Temperatures

During the abovementioned investigation of the reaction between hydrogen sulphide and ozonized oxygen, it was necessary to condense, at −183 °C, unreacted hydrogen sulphide with sulphur dioxide, a product of the reaction. It was then planned to store the mixed condensate in sealed tubes for subsequent analysis, and accordingly, since the condensation was made under anhydrous conditions, it was somewhat surprising to find a coherent film of plastic sulphur on the walls of the glass tubes, after they had been stored at room temperature.

Further experiments revealed that when a liquid hydrogen sulphide-sulphur dioxide mixture, contained in a sealed, previously outgassed (10^{-5} mm Hg) Pyrex storage tube of approximately 5 ml capacity, was slowly warmed from −78 °C, the liquid remained clear until about −40 °C, at which temperature a pale yellow deposit appeared on the walls of the tube just above the liquid level. As the temperature was slowly increased, deposition continued, and at about −20 °C a yellow precipitate was observed in the liquid remaining in the tube Deposition of the yellow film on the tube walls continued as the tube warmed to room temperature. This experiment was repeated many times, and invariably the same reaction pattern was closely followed. It is interesting to notice that these observations differ only from those of Quam, if it is assumed that lack of reaction observed by both Antony and Magri and later by Mathews arose because these workers maintained the temperature of their reactants below −40 °C. However, it must also be remembered that, in the present work, the use of sealed tubes leads ultimately to pressures in excess of 1 atm, which may also influence the course of the reaction.

A quantitative investigation of the reacting ratios of hydrogen sulphide to sulphur dioxide was undertaken by condensing (at −183 °C) a known amount of hydrogen sulphide with an excess of sulphur dioxide into a small evacuated Pyrex tube, which was then sealed under high vacuum. The tube and its contents were alternately warmed to room temperature and then recooled to −183 °C a number of times to ensure complete reaction between the entrapped

materials. The volatile contents of the tube were then transferred under vacuum to a low temperature fractionating column of the type described by Le Roy (1950), and carefully fractionated using take-off pressures between 0·001 and 1 mm. The initial concentrations of the reactants, the relative amounts recovered after reaction, and the reacting ratios for two typical experiments are given in Table 1.

The data contained in columns 3 and 4 clearly demonstrate that hydrogen sulphide reacts quantitatively (to *c.* 1 per cent.) with an excess of sulphur dioxide, and that with the exception of water vapour the residual sulphur dioxide is the only volatile material remaining after the reaction.* When the contents of the tube were cooled to −183 °C and the tube was broken, no pressure was developed confirming that hydrogen and oxygen were not products of the reaction. Further-

TABLE 1

REACTION BETWEEN HYDROGEN SULPHIDE AND SULPHUR DIOXIDE

Initial*		Recovered*		Reacted*		Reacting Ratio $H_2S : SO_2$
H_2S	SO_2	H_2S	SO_2	H_2S	SO_2	
1·39	1·56	0·02	0·65	1·37	0·91	3·02 : 2
2·68	2·30	0·03	0·54	2·65	1·76	3·01 : 2
Mean $H_2S : SO_2$						3·02 : 2

* g-mol $\times 10^4$.

more, it is clear from the data in columns 5, 6, and 7, that under the present reaction conditions, hydrogen sulphide and sulphur dioxide react in a mole ratio of 3 : 2. This contrasts with the reaction generally quoted in the literature for their reaction either in aqueous solution or in the moist gaseous phase, that is,

$$2H_2S+SO_2=2H_2O+3S.$$

Since the above study emerged as a side issue to the main investigation, only a superficial examination of the reaction products has so far been attempted. However, in addition to sulphur and water, an oxide or oxy acid of sulphur appears to be formed, for the aqueous extract of the non-volatile reaction products is strongly acidic.

It is difficult to speculate on the mechanism of the reaction in the absence of more detailed information. Even so, it is relevant to note that the melting point diagram of Biltz and Bräutigam (1927) for anhydrous mixtures of hydrogen sulphide and sulphur dioxide displays no maxima corresponding to compound formation, but only a eutectic at −110 °C which corresponds to a melt of composition $3H_2S \cdot SO_2$. Unfortunately, these workers did not report any data for the composition region 50–75 mole per cent., so that the existence of an unstable addition compound, $3H_2S \cdot 2SO_2$, cannot be excluded.

* Under the chosen reaction conditions, the reaction product believed to be water (on the basis of vapour pressure measurements), is not sufficiently volatile to be transferred from the Le Roy still at the take-off temperatures.

III. A Method for the Analysis of Milligram Quantities of Hydrogen Sulphide in the Presence of Carbon Dioxide

During the previously mentioned investigation of the reaction between hydrogen sulphide and ozonized oxygen, it became necessary to devise a method for analysing milligram quantities of hydrogen sulphide in the presence of traces

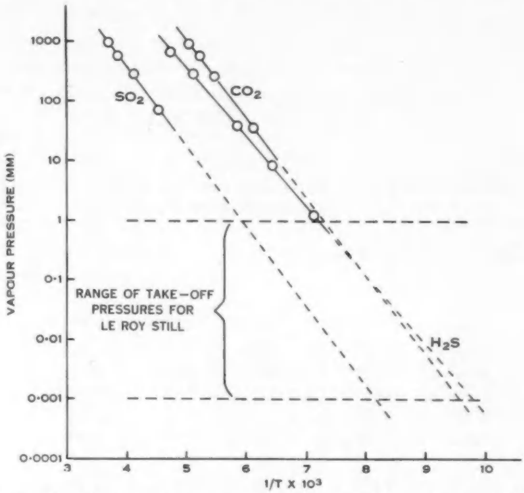

Fig. 1.—Vapour pressure of sulphur dioxide (Farkas and Melville 1939, p. 106), carbon dioxide (Farkas and Melville 1939, p. 108), and hydrogen sulphide (Clark, Cockett, and Eisner 1951).

of carbon dioxide, at the same time employing conventional low temperature and high vacuum fractionation methods. The volatilities of hydrogen sulphide and carbon dioxide (cf. Fig. 1) are so similar that it was found impossible to effect

Table 2

Vacuum Fractionation of a Carbon Dioxide and Sulphur Dioxide Mixture of Known Composition

Components	CO_2	SO_2	Volume of Sample (ml)
Known composition (%)	25·4	74·6	3·63 (760 mm/25 °C)
Found (%)	25·6	74·5	

their separation by means of a Le Roy fractionating column. On the other hand, the Le Roy column fractionates efficiently a mixture of sulphur dioxide and carbon dioxide (cf. Fig. 1) as shown by the typical analytical data contained in Table 2. Accordingly, a novel method for estimating hydrogen sulphide in the

presence of carbon dioxide was devised, using the hydrogen sulphide-sulphur dioxide reaction described in Section II as its basis. In this method, a measured excess of sulphur dioxide is condensed into a small evacuated tube (of volume *c.* 5 ml) with the hydrogen sulphide-carbon dioxide mixture to be analysed. The tube is sealed under high vacuum, and then alternately warmed to room temperature and cooled to −183 °C, a number of times to ensure that reaction between hydrogen sulphide and sulphur dioxide is complete. The residual gas (containing carbon dioxide and unreacted sulphur dioxide) is transferred to a Le Roy column and fractionated under high vacuum to determine the amounts of carbon dioxide and unreacted sulphur dioxide. A series of typical results obtained by this method is illustrated in Table 3.

TABLE 3

REACTION BETWEEN HYDROGEN SULPHIDE AND SULPHUR DIOXIDE IN THE PRESENCE OF CARBON DIOXIDE

Initially*		Recovered*		Reacted*		Reacting Ratio $H_2S : SO_2$
H_2S+CO_2	SO_2 Added	CO_2	SO_2	H_2S	SO_2	
1·20	1·46	0·29	0·86	0·91	0·60	3·04 : 2
1·48	1·89	0·32	1·09	1·16	0·80	2·90 : 2
2·70	2·55	0·16	0·87	2·54	1·68	3·02 : 2
2·17	2·12	0·18	0·86	1·99	1·26	3·16 : 2
5·13	5·35	0·13	1·98	5·00	3·37	2·97 : 2
0·97	1·49	0·60	1·23	0·37	0·26	2·85 : 2
1·63	1·49	0·34	0·65	1·29	0·84	3·07 : 2
0·75	1·85	0·23	1·48	0·52	0·37	2·82 : 2
Mean $H_2S : SO_2$ ..	..	..	..	..	..	2·98 : 2

* g-mol × 10^4.

Again, the most striking feature emerging from the experimental data is the constancy of the reacting ratio of hydrogen sulphide to sulphur dioxide, the mean value from this series of eight analyses being 2·98 : 2, in excellent agreement with the results presented in Table 1.

IV. EXPERIMENTAL

Samples of carbon dioxide, sulphur dioxide, and hydrogen sulphide were prepared by well-established methods, further purified by standard chemical means, and then repeatedly fractionated under high vacuum, until fractions were obtained with vapour pressures agreeing with those in Figure 1.

All experiments were made in an all-purpose "Stock-type" high vacuum apparatus. The low temperature fractionations of small polycomponent gas samples were performed in the ingenious low pressure column already described at length by Le Roy (1950). A particular advantage of this column for the present work, with milligram quantities of gas, is the negligible hold-up during fractionation.

V. Acknowledgments

The authors thank the Metropolitan Water Board of Sydney for financial assistance with this project.

VI. References

Antony, U., and Magri, G. (1905).—*Gazz. chim. ital.* **35** (i): 206.
Biltz, W., and Braütigam, M. (1927).—*Z. anorg. Chem.* **162**: 49.
Clark, A. M., Cockett, A. H., and Eisner, H. S. (1951).—*Proc. Roy. Soc.* A **209**: 408.
Farkas, A., and Melville, H. W. (1939).—"Experimental Methods in Gas Reactions." (MacMillan & Co.: London.)
Lang, W. R., and Carson, C. M. (1905).—*Proc. Chem. Soc.* **21**: 158.
Le Roy, D. T. (1950).—*Canad. J. Res.* B **28**: 492.
Mathews, E. (1926).—*J. Chem. Soc.* **1926**: 2270.
Quam, G. N. (1925).—*J. Amer. Chem. Soc.* **47**: 103.

REACTIONS OF TERTIARY BUTYL HYPOCHLORITE

I. THE CHLORINATION OF AROMATIC NITROAMINES*

By K. H. PAUSACKER†‡ and J. G. SCROGGIE†

[*Manuscript received April 6, 1959*]

Summary

Tertiary butyl hypochlorite reacts with 2-nitroaniline, 4-nitroaniline, *N*-methyl-4-nitroaniline, and *NN*-dimethyl-4-nitroaniline to form excellent yields of nuclear-chlorinated derivatives. Direct oxidation, to form azo compounds, is also observed with 4-nitroaniline and the yield of azo compound increases at higher temperatures. Demethylation also occurs when *N*-methyl-4-nitroaniline and *NN*-dimethyl-4-nitroaniline are used.

I. INTRODUCTION

It is well established that 2-nitroaniline reacts with sodium hypochlorite in alkaline solution to form benzofurazan oxide (Green and Rowe 1912*a*), whereas 2,2′-dinitroazobenzene is formed in neutral solution (Green and Rowe 1912*b*). On the other hand, hypochlorous acid reacts with the amine to form either 4,6-dichloro-2-nitroaniline in dilute hydrochloric acid solution (Seyewetz and Chaix 1927) or *NN*-dichloro-2-nitroaniline in ether solution at −20 °C (Goldschmidt and Strohmenger 1922). The *NN*-dichloro-derivative is converted into 4,6-dichloro-2-nitroaniline on standing in ether, but 2,2′-dinitroazobenzene is formed when carbon tetrachloride is the solvent.

As it was known that certain aromatic amines undergo chlorination by reaction with tertiary butyl hypochlorite (TBH) (Clark 1931; Anbar and Ginsburg 1954), it was decided to investigate the reaction of this compound with various nitroanilines in order to compare the products with those obtained with the above reagents.

II. EXPERIMENTAL

Analyses were made by the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory.

(*a*) *Reagents*

The amines were crystallized several times from appropriate solvents and thoroughly dried before use. The m.p.'s of the purified compounds were as follows: 2-nitroaniline (water), 72 °C; 4-nitroaniline (benzene), 148 °C; *N*-methyl-4-nitroaniline (ethanol), 152 °C; *NN*-dimethyl-4-nitroaniline (benzene or ethanol), 163 °C. TBH was prepared (Teeter and Bell 1952) either in diffuse daylight or in complete darkness and was stored in the dark. It was freshly distilled

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at least twice prior to use, b.p. 79–80 °C/760 mm, and the minimum purity (estimated iodometrically) was 96%. Benzene was purified according to Vogel (1948). Acetic acid was purified according to Eichelberger and La Mer (1933). *tert.*-Butanol was dried over freshly-ignited calcium oxide and then fractionated (Weissberger *et al.* 1955).

(*b*) *Reaction of Nitroanilines with TBH*

A solution of TBH in either benzene, *tert.*-butanol, or acetic acid was added (5–10 min) to a similar solution of the amine, either in the dark or in diffuse daylight, and the reaction was allowed to proceed to completion (iodometric estimation). The various conditions are summarized in Table 1. Any precipitate which formed was filtered off, the filtrate was evaporated to dryness, and the residue was dissolved in benzene and chromatographed on alumina, using benzene as eluent.

TABLE 1

EXPERIMENTAL CONDITIONS FOR THE REACTION OF TBH WITH VARIOUS AROMATIC NITROAMINES

Expt. No.	Amine	Weight of Amine (g)	Solvent	Volume of Solvent (ml)	TBH*	Temp. (°C)	Time (days)
1	2-Nitroaniline	2·80	Benzene	50	1·12	20	3
2		2·80	*tert.*-Butanol	50	1·12	20	1
3		2·80	Acetic acid	50	1·12	20	1
4	4-Nitroaniline	7·00	Benzene	1000	1·07	20	3
5		7·00	Benzene	1000	1·00	20	3
6		1·40	Benzene	200	2·16	20	1
7		2·80	Benzene	200	1·08	80	0·04
8		7·00	Benzene	500	6·05	80	0·20
9, 10, 11		—	Benzene	—	2–3	80	0·20
12		2·80	*tert.*-Butanol	100	2·16	80	0·02
13		2·80	Acetic acid	150	1·08	20	1
14		2·80	Acetic acid	150	2·16	20	1
15	*N*-Methyl-4-nitroaniline	1·52	Benzene	100	1·14	20	0·16
16		1·50	Benzene	100	2·22	20	1
17	*NN*-Dimethyl-4-nitroaniline	4·00	Benzene	250	1·09	20	1
18		3·00	Benzene	200	2·23	20	2
19	2-Chloro-4-nitroaniline	1·70	Benzene	120	1·25	80	0·33

* Mol/mol of amine.

(i) *Reaction with 2-Nitroaniline.*—6-Chloro-2-nitroaniline (m.p. and mixed m.p. with an authentic specimen, 76 °C) was obtained in the first chromatographic fraction from experiment Nos. 1, 2, and 3 in 38, 25, and 29% yield respectively. 4-Chloro-2-nitroaniline (m.p. and mixed m.p. with an authentic specimen, 116 °C) was obtained in the second fraction in 56, 75, and 64% yield respectively.

(ii) *Reaction with 4-Nitroaniline.*—The order in which the various products were eluted during chromatography was 2,2′-dichloro-4,4′-dinitroazobenzene (m.p. and mixed m.p. with an authentic specimen, 267 °C), 4,4′-dinitroazobenzene (m.p. and mixed m.p. with an authentic

specimen, 225 °C), 2,6-dichloro-4-nitroaniline (m.p. and mixed m.p. with an authentic specimen, 190 °C), and 2-chloro-4-nitroaniline (m.p. and mixed m.p. with an authentic specimen, 108 °C). The percentage yields of the various products are shown in Table 2.

Chromatography of the products from experiment number 8 yielded (i) an orange-red substance (1·11 g) which crystallized from *cyclo*hexane and had m.p. 120–150 °C ; repeated chromatography and crystallization failed to purify this substance further ; (ii) a red-brown substance (3·36 g) (compound A) which crystallized from benzene, ethanol, or *cyclo*hexane and had m.p. 190 °C (Found : C, 48·9 ; H, 2·5 ; Cl, 8·2 ; N, 18·7 ; O, 20·9%) ; a mixed m.p. with 2,6-dichloro-4-nitroaniline was <160 °C ; (iii) 2,2′-dichloro-4,4′-dinitroazobenzene (0·064 g) (Found : C, 42·4 ; H, 1·9 ; Cl, 20·1 ; N, 16·3%. Calc. for $C_{12}H_6Cl_2N_4O_4$: C, 42·2 ; H, 1·8 ; Cl, 20·8 ; N, 16·4%) and (iv) 4,4′-dinitroazobenzene (1·75 g).

The products of several other similar reactions (experiments Nos. 9, 10, and 11) were roughly separated by ether-extraction of the crude product, followed by crystallization. The percentage yields (calculated on a weight basis only) of compound A were 44, 42, and 38% respectively, while 4,4′-dinitroazobenzene was obtained in 47, 33, and 38% yield respectively. Small amounts (up to 2·5%) of 2,2′-dichloro-4,4′-dinitroazobenzene were again isolated.

Table 2

REACTION OF TBH WITH 4-NITROANILINE

Expt. No.	4,4′-Dinitro-azobenzene	2,2′-Dichloro-4,4′-dinitroazobenzene	2,6-Dichloro-4-nitroaniline	2-Chloro-4-nitroaniline
4	1	—	6	92
5	3	—	—	94
6	0·4	—	91	—
7	8	—	4	79
12	—	0·6	82	—
13*	—	—	7	84
14	—	—	79	—

* A precipitate (0·062 g) was formed during the reaction and, although it was insoluble in most common solvents, it could be crystallized from nitrobenzene and had m.p. >300 °C. The material formed in two experiments had the same analysis (Found : C, 46·0 ; H, 3·0 ; Cl, 17·6 ; N, 15·3 ; O, 18·1%). This compound (0·100 g) was refluxed (1 hr) with hydrochloric acid (11N ; 4 ml) in ethanol (10 ml) and, after distillation of the ethanol and subsequent neutralization, the solution gave 2-chloro-4-nitroaniline (0·090 g ; m.p. and mixed m.p. with an authentic specimen, 107 °C).

The various samples of compound A always had the same m.p. (190 °C) but consistent analyses could not be obtained. It could not be further purified by repeated chromatography (on alumina or on "Zeo-Karb 225" ion-exchange resin), fractional crystallization, vacuum-sublimation, or vacuum-distillation, and was recovered after refluxing with acetic anhydride–pyridine.

(iii) *Reaction with* N-*Methyl-4-nitroaniline.*—In experiment No. 15, 2-chloro-*N*-methyl-4-nitroaniline (93% yield), m.p. 118 °C (lit. m.p. 116 °C) (Found : C, 45·3 ; H, 4·2%. Calc. for $C_7H_7ClN_2O_2$: C, 45·0 ; H, 3·8%), was obtained in the first chromatographic fraction and 2-chloro-4-nitroaniline (7%), m.p. and mixed m.p. with an authentic specimen, 108 °C, was obtained in the second fraction.

In experiment No. 16, *2,6-dichloro-*N*-methyl-4-nitroaniline* (90% yield), m.p. 70 °C (from *cyclo*hexane) (Found : C, 38·2 ; H, 2·6%. Calc. for $C_7H_6Cl_2N_2O_2$: C, 38·0 ; H, 2·7%) was obtained in the first fraction and 2-chloro-4-nitroaniline (5%), m.p. and mixed m.p. 107 °C, was obtained in the second fraction.

(iv) *Reaction with* NN-*Dimethyl-4-nitroaniline.*—In experiment No. 17, small droplets of a colourless liquid were precipitated from the reaction mixture. This liquid was soluble in water

and had a marked odour of formic acid. Chromatography of the products yielded (i) 2-chloro-*NN*-dimethyl-4-nitroaniline (93% yield), m.p. 76 °C (lit. m.p. 78 °C) (Found : C, 48·3 ; H, 4·9%. Calc. for $C_8H_9ClN_2O_2$: C, 47·9 ; H, 4·5%) ; (ii) unchanged *NN*-dimethyl-4-nitroaniline (0·080 g) ; (iii) *N*-methyl-4-nitroaniline (3%), m.p. and mixed m.p. with an authentic specimen, 151 °C ; and (iv) a very tightly-adsorbed yellow band which was extracted with boiling ethanol to give a yellow solid (0·137 g). This solid (m.p. >260 °C) was insoluble in the common solvents and appeared to be an aluminium complex ; on treatment with hydrochloric acid, 2,6-dichloro-4-nitrophenol, m.p. and mixed m.p. with an authentic specimen, 128 °C, was obtained. Chromatography of the original starting material revealed no trace of a phenolic impurity.

In experiment No. 18, small droplets of a colourless liquid were again deposited ; this liquid gave a positive test for formic acid with resorcinol–sulphuric acid. Chromatography of the products yielded (i) *2,6-dichloro-*NN*-dimethyl-4-nitroaniline* (69% yield), m.p. 103 °C (from ethanol) (Found : C, 40·8 ; H, 3·3%. Calc. for $C_8H_8Cl_2N_2O_2$: C, 40·8 ; H, 3·4%) ; (ii) 2-chloro-*N*-methyl-4-nitroaniline (7%), m.p. and mixed m.p. with an authentic specimen, 116 °C ; and (iii) a very tightly-adsorbed yellow band which yielded the same aluminium complex (0·500 g) as obtained in experiment No. 17.

(v) *Reaction with 2-Chloro-4-nitroaniline.*—In experiment No. 19, 2,2′-dichloro-4,4′-dinitroazobenzene (0·322 g), m.p. and mixed m.p. with an authentic specimen, 269 °C, crystallized from the cooled reaction mixture. The other products of the reaction were not investigated.

(c) *Oxidation of 2-Chloro-4-nitroaniline with Iodosobenzene Diacetate*

2-Chloro-4-nitroaniline (1·70 g) was treated with a solution of iodosobenzene diacetate (5·00 g) in benzene (100 ml) at 35 °C. The solution became red-brown and reaction was complete in 1 day. The red-brown precipitate (0·670 g, m.p. 265 °C) was filtered off and the filtrate was chromatographed on alumina. Elution with benzene gave 2,2′-dichloro-4,4′-dinitroazobenzene (0·804 g), m.p. 269 °C, undepressed on admixture with the above precipitate. This product was used in the mixed m.p. determinations described in Section II (*b*) (ii).

III. Discussion

6-Chloro-2-nitroaniline (25–38 per cent.) and 4-chloro-2-nitroaniline (75–56 per cent.) were formed when 2-nitroaniline reacted with 1 mol of TBH in *tert*.-butanol, acetic acid, or benzene. Thus TBH acts as a chlorinating agent, like hypochlorous acid, and does not resemble sodium hypochlorite which acts as an oxidizing agent in either alkaline or neutral solution. The relative yield of the 4- and 6-chloro compounds was dependent on the solvent, but no convincing reason can be given for the variation. Only 4-chloro-2-nitroaniline has been reported as a product when 2-nitroaniline reacts with *N*,2,4-trichloroacetanilide (Chattaway and Orton 1901) or diethyl *N*-chloroiminocarbonate (Likhosherstov, Petrov, and Voskresenskaya 1935).

Similarly, 4-nitroaniline reacts with either 1 or 2 mol of TBH in benzene or acetic acid, at room temperature, to form either 2-chloro-4-nitroaniline or 2,6-dichloro-4-nitroaniline respectively in excellent yield. In benzene, small yields (0·5–3·0 per cent.) of 4,4′-dinitroazobenzene were formed simultaneously, and it is known that this compound is the principal product when 4-nitroaniline is treated with certain oxidizing agents (Barlin, Pausacker, and Riggs 1954 ; Pausacker and Scroggie 1954). In experiment No. 7, the reaction was performed at 80 °C and the yield of the azo compound (8 per cent.) was substantially increased. When an excess of TBH was allowed to react with 4-nitroaniline in benzene at 80 °C, much higher yields (38–47 per cent.) of the azo compound were obtained, as well as appreciable amounts of an unidentified substance,

m.p. 190 °C (see Section II). In addition, small amounts (up to 2·5 per cent.) of 2,2′-dichloro-4,4′-dinitroazobenzene were isolated in these last experiments. This compound is presumably formed by oxidation of the 2-chloro-4-nitroaniline resulting from initial chlorination of 4-nitroaniline, as it was obtained in higher yield when 2-chloro-4-nitroaniline was treated similarly, and it was shown that 4,4′-dinitroazobenzene does not react with TBH under such conditions.

When the reaction of 4-nitroaniline with TBH was carried out in boiling *tert.*-butanol, instead of benzene, chlorination was again the predominant reaction, although a small yield (0·6 per cent.) of 2,2′-dichloro-4,4′-dinitroazobenzene was isolated.

In contrast, it was found that 4-nitroacetanilide would not react with TBH in either (i) benzene at room temperature or 80 °C, or (ii) acetic acid at room temperature.

Chlorination was again the principal reaction when *N*-methyl-4-nitroaniline was treated with either 1 or 2 mol of TBH in benzene solution, but some 2-chloro-4-nitroaniline was also formed in each case. Thus some demethylation occurred and this effect has been observed frequently during the reaction of *N*-methylanilines with certain oxidizing agents (cf. Mitchell and Pausacker 1957).

NN-Dimethyl-4-nitroaniline reacted with 1 mol of TBH in benzene to yield 2-chloro-*NN*-dimethyl-4-nitroaniline, but demethylation also occurred as *N*-methyl-4-nitroaniline and formic acid were isolated. When 2 mol of TBH was used, the principal product was 2,6-dichloro-*NN*-dimethyl-4-nitroaniline but demethylation products (2-chloro-*N*-methyl-4-nitroaniline and formic acid) were once again formed. In both reactions, there was a very tightly adsorbed band, which could be extracted with boiling ethanol, on the alumina column. The product appeared to be an aluminium complex and, when treated with hydrochloric acid, it was converted into 2,6-dichloro-4-nitrophenol. Chlorination of the nucleus evidently precedes the replacement of the dimethylamino group, as it was found that 4-nitrophenol would not react with TBH in benzene at room temperature ; it is possible that this product could arise from a reaction at basic centres in the alumina.

In order to establish the mechanism of the major chlorination reactions, it was decided to investigate the rate of reaction of the various 4-nitroanilines with TBH under differing conditions. This study will be presented in a subsequent paper.

IV. Acknowledgments

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V. References

Anbar, M., and Ginsburg, D. (1954).—*Chem. Rev.* **54** : 925.
Barlin, G. B., Pausacker, K. H., and Riggs, N. V. (1954).—*J. Chem. Soc.* **1954** : 3122.
Chattaway, F. D., and Orton, K. J. P. (1901).—*J. Chem. Soc.* **79** : 461.
Clark, B. F. (1931).—*Chem. News* **43** : 265.
Eichelberger, W. C., and La Mer, V. K. (1933).—*J. Amer. Chem. Soc.* **55** : 3633.
Goldschmidt, S., and Strohmenger, L. (1922).—*Ber. dtsch. chem. Ges.* **55** : 2450.

GREEN, A. G., and ROWE, F. M. (1912*a*).—*J. Chem. Soc.* **101** : 2452.
GREEN, A. G., and ROWE, F. M. (1912*b*).—*J. Chem. Soc.* **101** : 2443.
LIKHOSHERSTOV, M. V., PETROV, A. A., and VOSKRESENSKAYA, L. N. (1935).—*Acta Univ. voroneg.* **8** : 57. (*Chem. Abstr.* **32** : 6633 (1938).)
MITCHELL, JOAN, and PAUSACKER, K. H. (1957).—*Aust. J. Chem.* **10** : 460.
PAUSACKER, K. H., and SCROGGIE, J. G. (1954).—*J. Chem. Soc.* **1954** : 4003.
SEYEWETZ, A., and CHAIX, E. (1927).—*Bull. Soc. chim. Fr.* **41** : 196.
TEETER, H. M., and BELL, E. W. (1952).—*Org. Synth.* **32** : 20.
VOGEL, A. I. (1948).—" A Textbook of Practical Organic Chemistry." p. 171. (Longmans: London.)
WEISSBERGER, A., PROSKAUER, E. S., RIDDICK, J. A., and TOOPS, E. E. (1955).—" Organic Solvents." p. 349. (Interscience Publishers Inc.: New York.)

TERPENOID CHEMISTRY

I. ZIERONE AND ELLERYONE

By R. P. Hildebrand* and M. D. Sutherland*

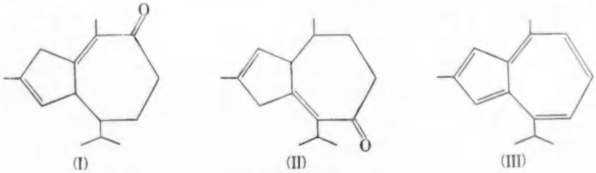
[*Manuscript received February 2, 1959*]

Summary

Elleryone, the sesquiterpene ketone from the essential oil of *Evodia elleryana* F. Muell., is now found to be identical with the known substance zierone. A revised structure for zierone is proposed.

I. Introduction

The ketone, zierone, isolated from the essential oil of *Zieria macrophylla* Bonpland by Penfold (1926), was shown to be an azulenogenic sesquiterpene ketone, $C_{15}H_{22}O$, by Bradfield, Penfold, and Simonsen (1933). Renewed investigation by Birch, Collins, and Penfold (1955) led to the provisional structures I or II based on the formation of zierazulene (III) by dehydrogenation.



(I) (II) (III)

An apparently new sesquiterpene ketone, elleryone, $C_{15}H_{22}O$, was isolated from *Evodia elleryana* F. Muell. by Jones and Wright (1946), and differed from zierone in the melting point of the semicarbazone and notably in the specific rotation of the ketone ($[\alpha]_D^{20}$ $-116°$ and $-118°$ for two different preparations).

II. The Identity of Elleryone with Zierone

A further preparation of elleryone from *E. elleryana* from Stradbroke I. was made by one of us with Mr. G. Lahey in 1946 and yielded a revised value for the optical rotation, namely, $[\alpha]_D^{26}$ $-150°$, which is comparable with that ($[\alpha]_D$ $-141 \cdot 2°$) reported by Penfold (1926) for zierone. However, at this stage the Kuhn and Roth (1932) *iso*propylidene estimation was applied to elleryone semicarbazone and yielded about $0 \cdot 7$ mole of acetone, an apparently clear distinction from zierone, which Bradfield, Penfold, and Simonsen (1933) reported as yielding no acetone by ozonolysis.

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More recently, examination of elleryone revealed ultraviolet absorption (maxima of 8380 and 1270 at 237 and 327 mμ in *cyclo*hexane) indicative of a highly substituted αβ-unsaturated carbonyl chromophore such as is present in zierone also. Careful comparison of the physical properties of the two ketones and their derivatives (including mixed melting points) has now revealed no significant differences. The infra-red spectra show virtually complete correspondence over the range 700 to 3500 cm^{-1}.

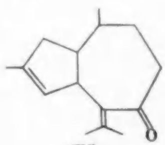
The only discrepancy, namely, the statement that zierone does not yield acetone on ozonolysis, has now been checked by ozonizing an authentic sample of zierone. In fact, zierone on ozonolysis gives acetone, isolated as the dinitrophenylhydrazone, in fair yield.

Zierone and elleryone are thus identical and the name elleryone (though used in this paper to distinguish zierone ex *E. elleryana* from zierone ex *Z. macrophylla*) has become redundant. The family Rutaceae includes both *Evodia* and *Zieria*.

III. A Revised Structure for Zierone

Although zierone semicarbazone gave an only fair yield (0·7 mole) of acetone on ozonolysis by the Kuhn-Roth procedure, a higher yield (0·87 mole) was obtained by extending the period of ozonation (to be discussed in a subsequent paper in this series). Moreover, ozonolysis of the ketone itself gave excellent yields (0·96 and 1·00 moles) by the Kuhn-Roth procedure and the hypoiodite-consuming material was positively identified as acetone by isolation as the dinitrophenylhydrazone.

The production of acetone from zierone necessitates modification of structures I and II proposed by Birch, Collins, and Penfold (1955) and, taken in conjunction with the other evidence, leads to two alternatives IV or V.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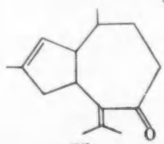
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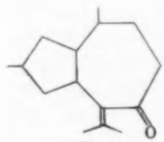
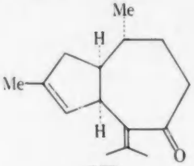

(VI)

The results of Birch, Collins, and Penfold (1955) restrict the carbonyl group to positions 5 or 7 of the zierazulene skeleton. As a consequence of the production of acetone on ozonolysis and the presence of an αβ-unsaturated carbonyl chromophore, the carbonyl group is further restricted to position 7. This necessitates the grouping $Me_2C{=}C{-}C{=}O$ in zierone, an arrangement which in pulegone (Wallach 1896), piperitenone (Naves 1942), and certain other terpenes, permits the liberation of acetone by an acid-catalysed hydrolysis. Zierone also, refluxed with 70 per cent. sulphuric acid, liberates acetone, isolated as the dinitrophenylhydrazone in 12 per cent. yield.

The other olefinic bond, restricted to either of two positions (IV or V) by the results of Birch, Collins, and Penfold (1955), can be selectively hydrogenated in the semicarbazone by the use of Adams's catalyst in alcohol, to yield a mixture

of stereoisomers, one only being crystallizable as an alcoholate. While the ultraviolet absorption ($\varepsilon_{max.}$ 9160 at 235 mμ) of this semicarbazone is, at first sight, anomalous for an αβ-unsaturated ketone (see later), the yield (91 per cent.) of acetone from ozonolysis, shows that the isolated olefinic bond is reduced, leading to VI for this dihydro compound.

Birch *et al.* (1957), taking this new evidence into account, have offered a probable solution (VII) to the structure and stereochemistry of zierone. This is based on the previous results and the interpretation that the exceedingly low extinction coefficient (ε 2540) of dihydrodihydroxyzierone (VIII) as compared with zierone itself (ε 7500) results from intramolecular hemiacetal formation as in IX.



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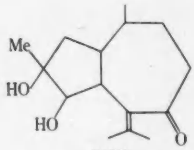

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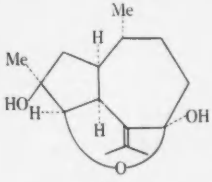

(IX)

The ultraviolet absorption of zierone (Fig. 1) ($\varepsilon_{max.}$ 8380 and 127 at 237 and 327 mμ in *cyclo*hexane) is of interest in that the αβ-unsaturated carbonyl chromophore represents a type not previously described in the literature, both bonds of the conjugated system being exocyclic to a seven-membered ring. Closely analogous is pulegone ($\varepsilon_{max.}$ 7950 and 50 at 244·5 and 331 mμ in hexane (Naves and Papazain 1942)), which differs in that the ring is six-membered. A hypsochromic shift with increased ring size is observed also on comparing 2,6-dibenzylidene *cyclo*hexanone ($\lambda_{max.}$ 319 mμ) with 2,7-dibenzylidene *cyclo*-heptanone ($\lambda_{max.}$ 290 mμ; Leonard and Robinson 1953). The α-diketones, 3,3,6,6,-tetramethyl*cyclo*hexadione ($\lambda_{max.}$ 380 mμ) and 3,3,7,7,-tetramethyl-*cyclo*heptadione ($\lambda_{max.}$ 337 mμ) provide a further example of such a hypsochromic shift, which Leonard and Mader (1950) attribute to the greater divergence from coplanarity of the two elements of the conjugated chromophore when arranged on a seven-membered ring.

Zierone semicarbazone ($\varepsilon_{max.}$ 11900 at 245 mμ in EtOH) and dihydrozierone semicarbazone ($\varepsilon_{max.}$ 9160 at 235 mμ in EtOH) both differ considerably in their ultraviolet absorption (Fig. 1) from typical saturated semicarbazones ($\lambda_{max.}$ 225–230 mμ) and from αβ-unsaturated semicarbazones ($\lambda_{max.}$ 259–273 mμ). For example, piperitone semicarbazone shows $\varepsilon_{max.}$ 19300 at 266 mμ. The observed absorption peaks must be interpreted as due to a combination of weakened and hypsochromically displaced K bands of the non-coplanar αβ-unsaturated semicarbazone chromophore and bands due to the partial unconjugated semicarbazone chromophore (Braude *et al.* 1949). That pulegone semicarbazone has $\varepsilon_{max.}$ 9400 at 256 mμ (in EtOH) suggests that in this simpler molecule also the conjugated chromophore is sufficiently distorted to show in some degree the characteristic wavelength and intensity effects.

The more extreme effect shown by dihydrodihydroxyzierone semicarbazone as compared with zierone semicarbazone, may follow from the increase in volume at positions 1 and 2 of the dihydro compound, causing a substantial increase in non-bonded interaction with the *iso*propylidene group. Alternatively, greater flexibility of the *cyclo*pentane ring as compared to the *cyclo*pentene ring may permit an adjusted conformation of the molecule in which further distortion of the chromophore is balanced against reduced non-bonded interaction (Braude and Sondheimer 1955).

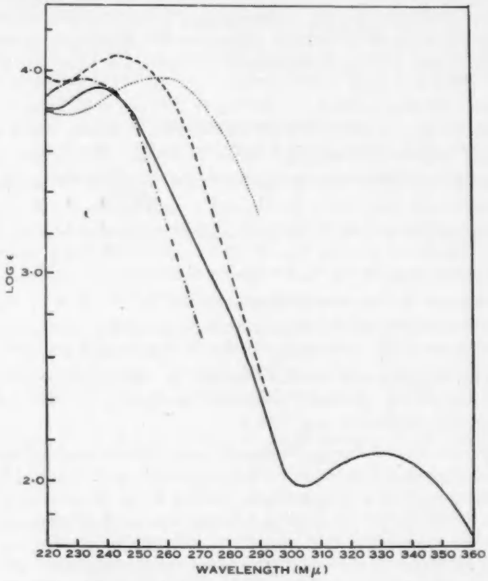

Fig. 1.—Ultraviolet absorption spectra.
—— Zierone in *cyclo*hexane.
- - - - Zierone semicarbazone in ethanol.
—·— Dihydrozierone semicarbazone in ethanol.
· · · · · Pulegone semicarbazone in ethanol.

Possibly the exceptionally low extinction coefficient of dihydroxyzierone might result not from hemiacetal formation but rather from either of the above factors causing still further distortion of the near *s-cis* arrangement of the chromophore of zierone itself. On this argument also, the 1,2-position of the isolated double bond would be the more probable.

IV. Experimental

(*a*) *Isolation of Elleryone Semicarbazone.*—A sample (15 lb) of *E. elleryana* leaves collected at Myora, Stradbroke I., was steam distilled for 45 hr to yield 51 ml of essential oil which on fractionation at 5 mm pressure yielded 6·6 g of a fraction of b.p. 122–126 °C/5 mm. This fraction yielded 5·1 g of crude crystalline semicarbazone of m.p. 178–181 °C, which by crystallization from

ethanol yielded pure elleryone semicarbazone of m.p. 182–183 °C (decomp.), $[\alpha]_D^{19}$ —146·4° (*c*, 2·67 in EtOH), $[\alpha]_D^{25}$ —136° (*c*, 2·3 in $CHCl_3$). The crystals have a very pale yellowish tinge which becomes more pronounced on exposure to light. The observed melting point varies with the rate of heating and temperature of immersion. Jones and Wright (1946) found m.p. 177–179 °C, $[\alpha]_D$ —144° (*c*, 2·1 in EtOH).

Zierone semicarbazone is reported by Penfold (1926) to have m.p. 180–181 °C, $[\alpha]_D^{20}$ —137° (*c*, 5·25 in $CHCl_3$). A mixed melting point test of elleryone semicarbazone with authentic zierone semicarbazone, m.p. 182–183 °C (decomp.), showed no depression.

(*b*) *Elleryone*.—The pure semicarbazone was hydrolysed by refluxing with an excess of 5% phthalic acid solution under an oil trap. After 2½ hr, 3·1 ml of oil (90% of the theoretical yield) was obtained from 4·05 g of semicarbazone. Fractionation of a larger quantity of regenerated ketone yielded, as a plateau fraction, colourless elleryone, b.p.$_2$ 109 °C, b.p.$_5$ 124 °C, b.p.$_{10}$ 138 °C, n_D^{25} 1·5117, d_4^{25} 0·9667, and $[\alpha]_D^{26}$ —150° (hom.). A *cyclo*hexane solution showed ultraviolet absorption maxima at 237 mμ (ε 8380) and 327 mμ (ε 127) and minima at 223 mμ (ε 6570) and 303 mμ (ε 89), with no fine structure in the long wavelength band. Jones and Wright (1946) report b.p.$_{0\cdot5}$ 70–72 °C, $n_D^{15\cdot5}$ 1·5163, $d_{15\cdot5}^{15\cdot5}$ 0·9750, and $[\alpha]_D^{20}$ —118°.

For zierone, Penfold (1926) found b.p.$_{10}$ 142·5–144 °C, d_{15}^{15} 0·9752, n_D^{20} 1·5142, and $[\alpha]_D$ —141·2°.

The 2,4-dinitrophenylhydrazone of elleryone could be obtained only by the use of ice-cold Brady's reagent, as orange-red needles, m.p. 95–96 °C. Bradfield, Penfold, and Simonsen (1933) report terracotta needles, m.p. 95–97 °C, for zierone dinitrophenylhydrazone.

The phenylhydrazone of elleryone, primrose needles, 107·5–108·5 °C, $[\alpha]_D^{25}$ —277° (*c*, 0·56 in $CHCl_3$) caused no depression of the melting point of authentic zierone phenylhydrazone for which m.p. 107–108 °C and $[\alpha]_D^{20}$ —279·5° (*c*, 5·65 in $CHCl_3$) has been reported by Penfold (1926).

Finally, elleryone, oxidized with osmium tetroxide by the technique used by Birch, Collins, and Penfold (1955) for zierone, yielded a crystalline product, m.p. 126 °C, closely comparable with their dihydroxydihydrozierone, m.p. 126 °C.

(*c*) *Partial Hydrogenation of Zierone Semicarbazone*.—Zierone semicarbazone (0·818 g) from *E. elleryana* when hydrogenated with Adams' catalyst in acetic acid, absorbed 1 mole of hydrogen, whereupon hydrogenation ceased. The resinous product taken up in ethanol yielded 0·37 g of large crystals, m.p. 96–99 °C, and on dilution a further 25 mg of product, m.p. 90–99 °C. The remaining solute could be isolated only as a gum. Crystallization of the solid product from ethanol yielded large chunky crystals of dihydrozierone semicarbazone alcoholate, m.p. 100–102 °C, $[\alpha]_D^{25}$ —163° (*c*, 1·2 in ethyl acetate) and $\varepsilon_{max.}$ 9200 at 235 mμ in 95% ethanol (Found: C, 67·0, 67·0; H, 10·6, 10·5; N, 13·1, 12·9%. Loss on heating at 80 °C *in vacuo* for 14 hr, 14·9%. Calc. for $C_{16}H_{27}N_3O.C_2H_5OH$: C, 66·9; H, 10·2; N, 13·0; C_2H_5OH, 14·3%).

The alcohol-free semicarbazone was a resin which could not be crystallized from any solvent but ethanol.

(*d*) *Acid Hydrolysis of Zierone*.—A stream of nitrogen gas was passed through 100 g of 70% sulphuric acid boiling under a reflux condenser and thence through 50 ml of a filtered solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid.

On the addition of 234 mg of zierone, the reaction mixture turned crimson and after 2 min, a yellow precipitate began to form in the dinitrophenylhydrazine solution. After ½ hr the solution was set aside. After 2 hr, the precipitate was recovered by filtration, washed with 25 ml of 2N hydrochloric acid and 25 ml of distilled water, and dried *in vacuo*. The dry substance, m.p. 100–120 °C, weighed 58·8 mg but after three crystallizations, the resulting 31·4 mg of dinitrophenylhydrazone had m.p. 124–124·5 °C and mixed m.p. 124–126 °C with authentic acetone dinitrophenylhydrazone (Found: C, 45·7; H, 4·2%. Calc. for acetone dinitrophenylhydrazone: C, 45·4; H, 4·2%).

(*e*) *Ozonolysis of Zierone*.—Zierone (182 mg) in 3 ml of acetic acid at room temperature was subjected to an ozone oxygen stream carrying 9 mg of ozone per min for 10 min (125% excess),

the issuing gas stream being washed by passage through ice-water. The wash water was added to the reaction solution which was distilled in a simple distilling apparatus to yield about 20 ml of distillate and collected in an ice-cooled vessel.

This distillate was diluted with 25 ml water, 1 ml acetic acid, and 0·4 g of potassium permanganate and was redistilled after boiling under reflux for 5 min. The distillate was passed directly into 50 ml of a saturated solution of dinitrophenylhydrazine in 2N hydrochloric acid. A yellow precipitate formed immediately and was treated as described in Section IV (*d*). The product, m.p. 117–123 °C, weighed 77·1 mg. One crystallization from ethanol gave 70·6 mg, m.p. 125–126 °C.

A number of determinations of acetone made under precipitation conditions approximating those described above showed losses of acetone dinitrophenylhydrazone due to incomplete precipitation, solubility in wash water, etc. ranging from 53·5 to 68·1 mg. Correcting for the smallest loss found, the yield of acetone dinitrophenylhydrazone (crude) becomes 130·6 mg or 66% of theory.

V. Acknowledgments

The authors thank Mr. H. G. McKern of the Museum of Applied Arts and Sciences for samples of authentic zierone and zierone semicarbazone, Mr. G. Lahey for valued assistance in obtaining a supply of elleryone semicarbazone, and the Australian Atomic Energy Commission for the award of a Research Studentship to one of us (R.P.H.).

VI. References

Birch, A. J., Collins, D. J., and Penfold, A. R. (1955).—*Chem. & Ind.* **1955**: 1773.

Birch, A. J., Collins, D. J., Hildebrand, R. P., Penfold, A. R., and Sutherland, M. D. (1957).—Paper presented at the Chemical Society Terpene Conference in July, 1957.

Bradfield, A. E., Penfold, A. R., and Simonsen, J. L. (1933).—*J. Roy. Soc. N.S.W.* **67**: 200.

Braude, E. A., Jones, E. R. H., Koch, H. P., Richardson, R. W., Sondheimer, F., and Toogood, J. B. (1949).—*J. Chem. Soc.* **1949**: 1890.

Braude, E. A., and Sondheimer, F. (1955).—*J. Chem. Soc.* **1955**: 3754.

Jones, T. G. H., and Wright, S. E. (1946).—*Pap. Dep. Chem. Univ. Qd.* **1**: No. 27.

Kuhn, R., and Roth, H. (1932).—*Ber. dtsch. chem. Ges.* **65**: 1285.

Leonard, N. J., and Mader, P. M. (1950).—*J. Amer. Chem. Soc.* **72**: 5394.

Leonard, N. J., and Robinson, G. C. (1953).—*J. Amer. Chem. Soc.* **75**: 2716.

Naves, Y-R. (1942).—*Helv. Chim. Acta* **25**: 732.

Naves, Y-R., and Papazian, G. (1942).—*Helv. Chim. Acta* **25**: 1023.

Penfold, A. R. (1926).—*J. Roy. Soc. N.S.W.* **60**: 104.

Wallach, O. (1896).—*Liebigs Ann.* **289**: 337.

STUDIES IN PLANT CHEMISTRY

I. THE ESSENTIAL OILS OF EUCALYPTUS CAESIA BENTH. AND E. TORQUATA LUEHM. AND THE STRUCTURE OF TORQUATONE

By R. C. Bowyer* and P. R. Jefferies*

[*Manuscript received February 23, 1959*]

Summary

A preliminary study of the essential oils of *Eucalyptus caesia* Benth. and *E. torquata* Luehm. has revealed the presence of a common ketonic substituent $C_{16}H_{24}O_4$. On the basis of degradative work described, the new substance, torquatone, has been assigned structure II, [2,4,6-trimethoxy-3,5-dimethyl]benzyl *iso*propyl ketone.

I. Introduction

The lack of information concerning the extensive essential oil flora of Western Australia has prompted us to carry out a survey of these oils with particular emphasis on the higher boiling components. Such work is greatly facilitated by using relatively low distillation temperatures possible with spinning band columns, and, with some care, the analytical gas chromatographic method.

Attention was directed at an early stage to the decorative coral gum *Eucalyptus torquata* Luehm. because of the high density of the oil. A preliminary study has revealed that the sample of oil is of the pinene-cineole type, containing in addition, eudesmol and about 25 per cent. of substance $C_{16}H_{24}O_4$, m.p. 41 °C, for which the name torquatone is proposed. Subsequently the essential oil of *E. caesia* Benth. was examined. The essential oil consisted of approximately 50 per cent. of torquatone. The lowest boiling fraction was essentially aromadendrene, and globulol was readily isolated from the sesquiterpene alcohols. Two minor components in the latter fractions are currently being examined.

II. The Structure of Torquatone

Torquatone, $C_{13}H_{15}O(OMe)_3$, is optically inactive and not attacked by mild oxidizing agents. The remaining oxygen atom was proved to be an unconjugated carbonyl group by its infra-red spectrum (cm^{-1}) 2834 OMe, 1701 C=O, 1588 and 1660 C_6H_6, and by preparation of a 2,4-dinitrophenylhydrazone ($\varepsilon_{max.}$ 25,400 at 361·5 mμ) which was obtained only after some hours standing. The spectrum in the ultraviolet (Fig. 1) confirms a benzene ring without carbonyl conjugation.

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Reduction to the alcohol took place smoothly with lithium aluminium hydride. Dehydration to the olefin $C_{13}H_{15}(OMe)_3$ was accomplished with phosphorus oxychloride in pyridine. The hydrocarbon exhibited absorption maxima (mμ) at 259·0 ($\varepsilon_{max.}$ 17,660) and 219·7 ($\varepsilon_{max.}$ 30,500), clearly showing conjugation of the ethylenic linkage with the benzene ring and implying the presence of carbonyl, β to the aromatic ring in torquatone.

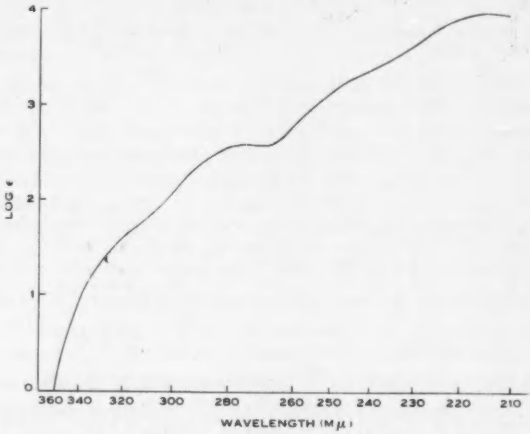


Fig. 1.—Ultraviolet spectrum of torquatone in ethanol.

Cold neutral permanganate was effective in rupturing the carbon chain of the hydrocarbon, and three products were isolated:

(i) A volatile acidic material, shown by paper chromatography to be a C_4 acid and proved to be *iso*butyric acid by characterization as the *p*-phenylphenacyl ester.

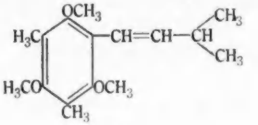
(ii) A non-volatile acid $C_{12}H_{16}O_5$, identified as 2,4,6-trimethoxy-3,5-dimethylbenzoic acid by comparison with a synthetic sample. These results are indicative of structure I for the hydrocarbon and II for torquatone.

(iii) A neutral oil which readily gave a 2,4-dinitrophenylhydrazone analysing as $C_{18}H_{20}N_4O_7$, presumably derived from 2,4,6-trimethoxy-3,5-dimethylbenzaldehyde.

That no rearrangement of the side chain occurred in the dehydration was indicated by the action of ethyl formate and potassium ethoxide on torquatone, which yielded a low-melting crystalline hydroxymethylene derivative, characterized as the aniline condensate and reconverted to torquatone by alkali hydroxide. This establishes the presence of a CH_2 group adjacent to the carbonyl, and adds further support to the structure II for torquatone.

Torquatone is thus another member of the phloroglucinol type to be isolated from the family Myrtaceae. The growing number of these substances and their

close relatives in this family is of some interest, and could possibly be of some taxonomic value, particularly in the difficult *Eucalyptus* classification. It is accordingly of interest to note that although some opinion has been to the contrary, Gardner (1931) has regarded *E. caesia* and *E. torquata* to be closely related botanically.



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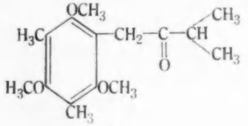

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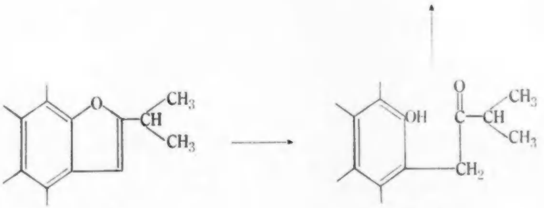

(III) (IV)

Biogenetically, torquatone is of interest since it is the first of the phloroglucinol types in the Myrtaceae in which the acyl moiety is not directly attached to the ring system and so is not immediately related to the biosynthetic routes covered by Birch (1957). (A possible mode of origin which would account for the β-carbonyl involves a direct introduction of the *iso*prenyl group as suggested by Birch (1957) for the humulone types, cyclization to III at some stage of synthesis, subsequent rupture, and methylation.)

A more complete investigation of the oils and synthesis of torquatone are in progress.

III. Experimental

(a) *General*

Analyses were carried out by the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory at the University of Melbourne. Melting points were determined in evacuated tubes and are uncorrected. Light petroleum refers to a fraction of boiling range 56–60 °C. Fractional distillations were carried out in a vacuum jacketed spinning band column of 30 theoretical plates (*n*-heptane, methyl*cyclo*hexane) using magnetic drive and incorporating a solenoid operated automatic take off.

Gas chromatography was carried out in a Griffin VPC Mk. II apparatus. Stationary phases included elastomer " Silicone E-301 " and " Reoplex 400 " (Geigy). The short life of the latter has led to its replacement by the tribenzoate of beyerol* which loses less than 1 mg/hr at a flow rate of 1 l/hr and 200 °C.

(b) *Isolation of the Oils*

(i) The oil (39 g) of *E. torquata* was obtained in 2·0% yield from steam-distillation in glass of leaves (1·95 kg) collected near Coolgardie in March 1957. Physical constants were $d_4^{20} = 0·952$; $n_D^{19} = 1·4820$; cineole = 36%.

* A tetracyclic diterpene triol from *Beyeria leschenaultia*.

(ii) Distillation of 17·2 kg of *E. caesia* leaves and terminal branches from Mt. Caroline in May 1957 gave 160 g (0·9%) of the oil. Collection of the oil on this scale necessitated a benzene trap, subsequent removal of which permitted some evaporation of lower-boiling constituents. The residual oil had $d_4^{20}=0\cdot994$.

A further distillation of fresh *E. caesia* (1130 g) yielded 8·5 g (0·75%) of the oil without the use of a scavenging solvent. This oil showed $d_4^{20}=0\cdot990$; $n_D^{22}=1\cdot4940$; $[\alpha]_D=+1°$.

(c) *Distillation of the Oils*

(i) *E. torquata* oil (39 g) was fractionated under reduced pressure, and the following groups of fractions collected:

(1) 65–70 °C/42 mm, 4·5 g; (2) 70–85 °C/42 mm, 10 g; (3) 60 °C/11 mm–134 °C/5·5 mm, 7 g; (4) 134–150 °C/5·5 mm, 5·1 g; (5) 150–157 °C/5·5 mm, 8·5 g. A typical fraction of group (1) showed $n_D^{20}=1\cdot4660$; $[\alpha]_D=+38\cdot3°$; $d_4^{15}=0\cdot890$. The physical constants correspond to those of α-pinene which was identified by its infra-red spectrum. Group (2) consisted essentially of cineole, the best fraction of which showed $n_D^{20}=1\cdot4605$; $[\alpha]_D=+2\cdot2°$; $d_4^{15}=0\cdot926$. The presence of 1,8-cineole was proved by the infra-red spectrum. Group (3) contained several unidentified substances each in small quantity. Group (4) solidified on standing and was recrystallized to constant m.p. 82–83 °C. It did not depress the melting point of an authentic sample of eudesmol and had the same infra-red spectrum. Group (5) crystallized, yielding *torquatone*.

(ii) *E. caesia* oil, in a typical fractionation of 62 g, gave the following groups of fractions:

(1) 116–124 °C/10 mm, 8·2 g; (2) 112–132 °C/7 mm, 7·0 g; (3) 132–145 °C/7 mm, 11·0 g; (4) 145–160 °C/7 mm, 6·1 g; (5) 160–162 °C/7 mm, 23·8 g; (6) dark residual oil, 4·2 g. A typical fraction of group (1) had physical constants $n_D^{21}=1\cdot4948$; $[\alpha]_D=+10\cdot7°$; $d_4^{21}=0\cdot920$, corresponding with those of aromadendrene. Ozonolysis of the oil yielded a crystalline solid, melting at 83·0–83·5 °C, alone or mixed with an authentic sample of *apo*aromadendrone. Group (2), containing intermediate fractions from (1) and (3), is receiving further attention for the identification of an additional constituent. Group (3) fractions solidified on standing, and after recrystallization yielded a colourless solid, m.p. 85–87 °C. The 3,5-dinitrobenzoate melted at 137–138 °C, and did not depress the melting point of an authentic sample of globulol 3,5-dinitrobenzoate. Group (4) contained further intermediates, and showed an unidentified gas chromatograph peak, intermediate between globulol and torquatone. Group (5) crystallized and was found to consist of torquatone. Group (6), the residue, yielded a further quantity of torquatone after distillation at 1 mm.

(d) *Properties of Torquatone*

The colourless crystalline solid had m.p. 40–41 °C after recrystallization of groups (5) of *E. torquata* and *E. caesia* fractionations from light petroleum (Found: C, 68·7; H, 8·6; O, 22·9; mol. wt. (after Rast), 271; OCH_3, 33·0%. Calc. for $C_{12}H_{15}O(OCH_3)_3$: C, 68·5; H, 8·6; O, 22·8; mol. wt., 280; OCH_3, 33·2%).

Torquatone-2,4-dinitrophenylhydrazone.—Torquatone (0·5 g) was treated with excess 2,4-dinitrophenylhydrazine reagent, and the derivative collected after 48 hr. Crystallization from ethanol gave orange plates, m.p. 202 °C (Found: C, 57·4; H, 6·1; N, 12·2%. Calc. for $C_{22}H_{28}N_4O_7$: C, 57·6; H, 6·0; N, 11·9%).

Hydroxymethylenetorquatone.—Potassium ethoxide (from 0·8 g of potassium metal) in dry ether (20 ml) was treated with torquatone (3·97 g) and dry ethyl formate (8 ml) and allowed to stand overnight. The reaction mixture was extracted with 2N sodium hydroxide and the aqueous layer acidified (HCl). Ether extraction yielded a viscous brown oil (1·40 g) which crystallized on standing. The product gave a deep wine colour with ethanolic ferric chloride, and an immediate precipitate with 2,4-dinitrophenylhydrazine reagent. The aniline condensate crystallized from light petroleum in colourless flakes, m.p. 124 °C (Found: C, 72·0; H, 7·6; N, 3·7%. Calc. for $C_{22}H_{29}NO_4$: C, 72·3; H, 7·6; N, 4·0%). Hydrolysis of hydroxymethylene torquatone with 2N sodium hydroxide overnight regenerated torquatone, m.p. 40 °C.

(e) Degradation of Torquatone

(i) *[2,4,6-Trimethoxy-3,5-dimethyl]benzyl* iso*Propyl Carbinol*.—Torquatone II (0·86 g) in anhydrous ether (80 ml) was treated with excess lithium aluminium hydride. After heating under reflux for 1 hr, the crude material was isolated in the usual way. Distillation under reduced pressure gave the alcohol (0·80 g), b.p. = 120 °C/0·1 mm, $n_D^{25} = 1{\cdot}5057$ (Found : C, 68·1 ; H, 9·1 ; O, 23·0%. Calc. for $C_{16}H_{26}O_4$: C, 68·1 ; H, 9·3 ; O, 22·7%).

(ii) *1-[2,4,6-Trimethoxy-3,5-dimethyl]phenyl-3-methylbutene* (*I*).—The above alcohol (2·0 g) in dry pyridine (40 ml) was treated with phosphorus oxychloride (5 ml) and heated for 8 hr on the steam-bath in a stoppered vessel. Isolation with ether and distillation of the crude product gave olefin I (1·5 g), b.p. 126 °C/2 mm, $n_D^{19} = 1{\cdot}5185$ (Found : C, 72·76 ; H, 8·96 ; O, 18·4%. Calc. for $C_{16}H_{24}O_3$: C, 72·7 ; H, 9·2 ; O, 18·2%).

(iii) *Permanganate Oxidation of I*.—Neutral permanganate oxidation of the olefin I was conducted in acetone solution at room temperature. Finely powdered potassium permanganate was added from time to time with agitation. When the colour persisted for several hours, the solvent was removed under reduced pressure, and the residue washed with ether to remove neutral material. Residual permanganate and manganese dioxide were decomposed with sodium oxalate and sulphuric acid, and the mixture steam-distilled. The acid distillate, neutralized with standard alkali, was evaporated to dryness, and a *p*-phenylphenacyl ester, m.p. 86–87 °C, prepared (Found : C, 76·9 ; H, 6·6%. Calc. for $C_{18}H_{18}O_3$: C, 76·6 ; H, 6·4%). The melting point of a mixture with the authentic ester of *iso*butyric acid was undepressed.

The non-volatile acid, after recrystallization from water, melted at 125 °C, and was found to be identical with 2,4,6-trimethoxy-3,5-dimethylbenzoic acid by melting point and mixed melting point comparison with the methyl ester (m.p. 49–50 °C) and the free acid (Found : C, 60·0 ; H, 6·6%. Calc. for $C_{12}H_{16}O_5$: C, 60·0 ; H, 6·7%). Authentic samples were prepared by the method of Robertson and Whalley (1951) except zinc cyanide replaced the hydrogen cyanide. Methylation to the trimethoxy ester with dimethyl sulphate and potassium carbonate, and aqueous saponification provided the required acid.

The neutral extract from oxidation gave a 2,4-dinitrophenylhydrazone, crystallizing from ethanol in red needles, m.p. 221 °C (Found : C, 53·4 ; H, 4·9 ; N, 13·7%. Calc. for $C_{18}H_{20}N_4O_7$: C, 53·5 ; H, 5·0 ; N, 13·9%).

IV. Acknowledgments

The authors are indebted to Professor D. E. White for his interest and encouragement and to Mr. C. A. Gardner and Mr. J. W. Green for identification of plants. One of us (R.C.B.) wishes to thank C.S.I.R.O. for a Studentship.

V. References

Birch, A. J. (1957).—*Fortschr. Chem. org. Naturst.* **14** : 186.

Gardner, C. A. (1931).—" Enumeratio Plantarum Australiae Occidentalis." p. 88. (Government Printer : Western Australia.)

Robertson, A., and Whalley, W. B. (1951).—*J. Chem. Soc.* **1951** : 3355.

SIMPLIFIED ANALOGUES OF LYSERGIC ACID

I. DERIVATIVES OF 1,2,3,4-TETRAHYDRO-2-NAPHTHYLAMINE

By J. Cymerman Craig,* B. Moore,* and E. Ritchie*

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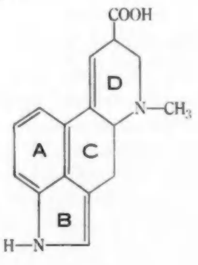
Summary

The preparation of certain *N*-alkylated derivatives of 1,2,3,4-tetrahydro-2-naphthylamine is described and their pharmacological activity is summarized.

I. Introduction

The pharmacological activity of the ergot alkaloids has been known since mediaeval times, but it is only in the last 20 years that their structures have been elucidated. All alkaloids of ergot have been shown to be amides of lysergic acid, which was first isolated by Smith and Timmis (1932) and Jacobs and Craig (1934*a*, 1934*b*). Its structure I was not definitely established until 1949 (Stoll, Hofmann, and Troxler 1949) and it was only in recent years that its total synthesis was achieved (Kornfeld *et al.* 1956).

Interest in the chemistry of lysergic acid was greatly stimulated, first by the discovery in 1947 (Stoll 1947) of the powerful psychiatric effects induced by lysergic acid diethylamide (LSD), and then by the observation by Gaddum (1953) of the intense antagonistic action of this substance to 5-hydroxytryptamine (5-HT, serotonin).



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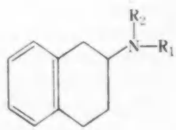

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The synthesis of simplified analogues of I was of interest in order to discover the smallest fragment of the polycyclic system present in the acid which would retain biological activity, and derivatives of 1,2,3,4-tetrahydro-2-naphthylamine (II, $R_1=R_2=H$) were chosen for examination. These may be regarded as constituting the structure of rings A and C of I but lacking ring B and with ring D opened.

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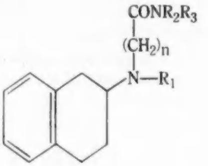
The synthesis of some related compounds has been examined by a number of workers. Marini-Bettolo, Chiavarelli, and Bovet (1952) and Marini-Bettolo, Vittory, and Bovet (1952) prepared amides of *N*-(1,2,3,4-tetrahydro-2-naphthyl)-β-alanine (III, $n=2$) and -glycine (III, $n=1$), and *N'*-substituted derivatives of *N*-(1,2,3,4-tetrahydro-2-naphthyl)diaminoethane (IV, $n=2$) and -propane (IV, $n=3$). Substances of type II, in which R_1 and R_2 were methyl and ethyl substituents, were synthesized by Chiavarelli *et al.* (1952). While all these substances displayed high sympatholytic activity, oxytocic action was shown by the amides of type III ($n=1$) and V.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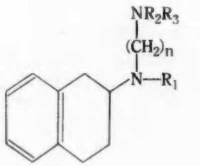
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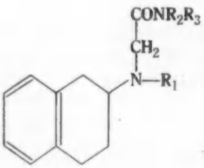
(V)

Kraushaar (1954) prepared a related series of 6-methoxy derivatives of II, in which R_1 and R_2 were methyl and 2-carboxyethyl substituents, with the parent compound ($R_1=R_2=H$) showing the strongest oxytocic action with the least side-effects.

Marini-Bettolo, Chiavarelli, and Bovet (1950) concluded that the 1,2,3,4-tetrahydro-2-naphthylamine element in the structure of the ergot alkaloids is more essential for the sympatholytic action than the indole moiety. It was accordingly decided to direct initial examination to substances of type II in which R_1 and R_2 were methyl, ethyl, propyl, and butyl substituents and various combinations thereof.

The amines were prepared by the appropriate combination of two reactions, methylation by formaldehyde-formic acid and acylation followed by reduction with lithium aluminium hydride. By the use of one molar proportion of formaldehyde in formic acid (II, $R_1=R_2=H$) gave the *N*-methyl derivative, which was converted by excess of the same reagent to the tertiary amine (II, $R_1=R_2=CH_3$). The acetyl derivative of II ($R_1=R_2=H$) was reduced by lithium aluminium hydride to II ($R_1=H$, $R_2=C_2H_5$) from which II ($R_1=CH_3$, $R_2=C_2H_5$) was obtained by the methylation procedure, and II ($R_1=R_2=C_2H_5$) by acetylation and reduction. In a similar manner the *n*-propyl, methyl-*n*-propyl, di-*n*-propyl, *n*-butyl, and *n*-butylmethyl derivatives were prepared. By catalytic hydrogenation of the Schiff's base from II ($R_1=R_2=H$) and *iso*butyraldehyde the branched chain derivative II ($R_1=H$, $R_2=CH_2CH(CH_3)_2$) was obtained, which was methylated as usual to the related tertiary amine.

An examination of these compounds has been carried out in the Department of Pharmacology of the University of Sydney by Miss J. N. Pennefather and Professor R. H. Thorp, to whom we are indebted for the results quoted below.

While the parent amine II ($R_1=R_2=H$) and its lower alkyl derivatives, for example, II ($R_1=R_2=CH_3$) exhibited pressor activity, the higher alkyl-

substituted compounds had hypotensive properties, and all substances tested showed effects on body temperature and respiration.

Similar results have been reported for the compounds II ($R_1=R_2=C_2H_5$) and II ($R_1=H$, $R_2=C_2H_5$) and their lower homologues by Bovet, Sollero, and Marotta (1952) and Bovet and Virno (1952). In these actions the synthetic compounds resembled the known effects of lysergic acid and the ergot alkaloids. Thus ergotamine produces a rise in blood pressure (Rothlin 1923), while the dihydro derivatives decrease it (Rothlin 1947); the ergot alkaloids also cause changes in body temperature (Rothlin 1947) and LSD is known to affect respiration (Rothlin 1957).

The action of the alkylated derivatives II on unanaesthetized cats resulted in a display of " sham rage " similar to that observed with the unsubstituted amine and with ergometrine by Brown and Dale (1935) and with LSD by Rothlin (1957). Marked antiserotonin activity was shown by II ($R_1=CH_3$, $R_2=n$-C_3H_7) which specifically antagonized the action of serotonin in a concentration of 1 part in 10 million (Pennefather and Thorp 1958).

It is seen that the compound most closely corresponding to the carbon skeleton of ring D in the intact lysergic acid molecule (I), that is, *N*-methyl-*N*-*n*-propyl-1,2,3,4-tetrahydro-2-naphthylamine, has shown itself to be the most interesting one in its high and specific antiserotonin activity, optimum adrenolytic action, and minimum depression of respiration (Pennefather and Thorp 1959). Further work in this field is proceeding.

II. Experimental

Melting points are uncorrected. Analyses were carried out by Dr. K. W. Zimmermann at the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory, and by Miss B. Stevenson, University of Sydney. Light petroleum refers to that fraction b.p. 60–90 °C.

(*a*) N-*Methyl-1,2,3,4-tetrahydro-2-naphthylamine*.—A solution of 1,2,3,4-tetrahydro-2-naphthylamine (5·0 g; 0·033 mole) in formic acid (10 ml) was treated with formalin (2·5 g of 40% solution; 0·033 mole) and the mixture heated under reflux until the evolution of carbon dioxide had ceased (4 hr). The solution was cooled, concentrated hydrochloric acid (10 ml) added, and the excess of formic acid and formalin removed *in vacuo* on the boiling water-bath. The residue was cooled, treated with sodium hydroxide solution (20%), and extracted with ether. Removal of solvent from the dried (anhydrous sodium sulphate) extracts and distillation of the residue gave the base, b.p. 84–85 °C/0·5 mm, n_D^{20} 1·5474, as a colourless oil (3·66 g, 70% yield). Waser (1916) reports b.p. 118–119·8 °C/9 mm. The hydrochloride crystallized from ethanol-ether as needles, m.p. 213–215 °C. Waser (1916) gives m.p. 214 °C, after sintering at 196 °C.

(*b*) NN-*Dimethyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Prepared from *N*-methyl-1,2,3,4-tetrahydro-2-naphthylamine (4·0 g; 0·025 mole), formic acid (8·0 ml), and formalin (2·5 g of 40%; 0·033 mole) by the method described in (*a*), the base (3·36 g, 77% yield) was obtained as a colourless oil, b.p. 95–96 °C/0·8 mm, n_D^{22} 1·5345. Waser (1916) reports b.p. 132·3–133·3 °C/11 mm. The hydrochloride which formed hygroscopic needles, m.p. 212–213 °C, from ethanol-ether, was converted by an absolute ethanolic solution of sodium bromide into the hydrobromide, crystallizing from ethanol-ethyl acetate as plates, m.p. 174–175 °C (Found: C, 56·3; H, 7·1%. Calc. for $C_{12}H_{18}NBr$: C, 56·3; H, 7·1%). Waser (1916) gives m.p. 214–215 °C (decomp.) for the hydrochloride.

(*c*) N-*Acetyl-1,2,3,4-tetrahydro-2-naphthylamine*.—A solution of 1,2,3,4-tetrahydro-2-naphthylamine (7·5 g; 0·05 mole) in dry pyridine (30 ml) was treated with acetic anhydride (12 ml). The mixture was heated on the boiling water-bath for 10 min., cooled, and poured onto crushed

ice. The acetyl compound (7·2 g, 75% yield) crystallized from benzene as needles, m.p. 107–108 °C. Cloetta and Waser (1913) give m.p. 108–108·5 °C.

(*d*) N-*Ethyl-1,2,3,4-tetrahydro-2-naphthylamine*.—The above acetyl compound (7·2 g; 0·038 mole) was placed in the thimble of a Soxhlet extractor, and extracted with dry ether (150 ml) containing lithium aluminium hydride (2 g; 0·052 mole) for 12 hr. After addition of wet ether and water, the mixture was extracted with ether, and the combined ether extracts shaken with dilute hydrochloric acid. The acid solution was basified with sodium hydroxide, and extracted with ether. Distillation of the ethereal extracts gave the amine (4·2 g, 63% yield) as an oil, b.p. 82–84 °C/0·3 mm, n_D^{24} 1·5340.

The hydrochloride crystallized from ethanol-ether as needles, m.p. 226–227 °C. Bamberger and Müller (1889) give b.p. 153 °C/23 mm for the base, and m.p. 223·5 °C for the hydrochloride.

(*e*) N-*Ethyl*-N-*methyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Reaction of *N*-ethyl-1,2,3,4-tetrahydro-2-naphthylamine (2·3 g), formic acid (5 ml), and formalin (2·5 g of 40%) by the method described in (*a*) gave the amine (2·1 g, 85% yield) as a colourless oil, b.p. 101–102 °C/0·9 mm, n_D^{22} 1·5312 (Found: C, 82·4; H, 10·2%. Calc. for $C_{13}H_{19}N$: C, 82·5; H, 10·1%).

The *hydriodide* crystallized from ethanol-ether in colourless needles, m.p. 125–127 °C (Found: C, 49·2; H, 6·4%. Calc. for $C_{13}H_{20}NI$: C, 49·2; H, 6·4%).

(*f*) N-*Acetyl*-N-*ethyl-1,2,3,4-tetrahydro-2-naphthylamine*.—*N*-Ethyl-1,2,3,4-tetrahydro-2-naphthylamine (5 g) was converted into the acetyl derivative (5·05 g, 81% yield), b.p. 162–164 °C/1 mm, by the method described in (*c*). Bamberger and Müller (1889) give b.p. 328 °C/718 mm.

(*g*) NN-*Diethyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Reaction of the above acetyl compound (5·05 g) with lithium aluminium hydride (1·5 g) in ether (40 ml) by the method described in (*d*) for 6 hr afforded the amine as an oil (3·71 g, 79% yield), b.p. 100–102 °C/0·5 mm, n_D^{20} 1·5280.

The *hydriodide* crystallized from ethanol-ether as needles, m.p. 138–139 °C (Found: C, 50·3; H, 6·7%. Calc. for $C_{14}H_{22}NI$: C, 50·7; H, 6·7%).

(*h*) N-*Propionyl-1,2,3,4-tetrahydro-2-naphthylamine*.—A solution of 1,2,3,4-tetrahydro-2-naphthylamine (7·5 g; 0·05 mole) in dry chloroform (30 ml) and dry pyridine (4·8 g; 0·06 mole) was treated with propionyl chloride (5·55 g; 0·06 mole) in chloroform (15 ml), and the mixture refluxed for 1 hr. After cooling, it was washed with dilute hydrochloric acid, sodium carbonate solution, and water, dried, and evaporated. The *product* crystallized from light petroleum as plates (8·35 g, 83% yield), m.p. 98–99 °C (Found: C, 76·5; H, 8·4%. Calc. for $C_{13}H_{17}ON$: C, 76·8; H, 8·4%).

(*i*) N-n-*Propyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Reaction of the above amide (13 g; 0·064 mole) and lithium aluminium hydride (3·5 g; 0·092 mole) in ether (200 ml) for 12 hr by the method described in (*d*) gave the amine as a colourless oil (9·3 g, 77% yield), b.p. 112–115 °C/1·5 mm, $n_D^{22\cdot5}$ 1·5302.

The *hydrochloride* crystallized from ethanol-ethyl acetate as needles, m.p. 242–243 °C (Found: C, 68·8; H, 8·8%. Calc. for $C_{13}H_{20}NCl$: C, 69·1; H, 8·9%).

(*j*) N-*Methyl*-N-n-*propyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Treatment of the above amine (2·3 g; 0·012 mole) with formic acid (4 ml) and formalin (1·4 g of 40%; 0·019 mole) by the method described in (*a*) for 3 hr afforded the tertiary amine (2·1 g, 85% yield) as a colourless oil, b.p. 110–111 °C/0·9 mm, n_D^{22} 1·5260.

The *hydriodide* separated from ethanol as needles, m.p. 144–145 °C (Found: C, 50·6; H, 6·6%. Calc. for $C_{14}H_{22}NI$: C, 50·7; H, 6·7%).

(*k*) N-*Propionyl*-N-n-*propyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Treatment of *N*-*n*-propyl-1,2,3,4-tetrahydro-2-naphthylamine (4·7 g; 0·025 mole) with propionyl chloride (3·5 g; 0·038 mole) by the method described in (*h*) for 2 hr, gave the *amide* (3·02 g, 50% yield) as a pale yellow oil, b.p. 168–171 °C/1·2 mm, n_D^{19} 1·5362 (Found: C, 77·7; H, 9·3%. Calc. for $C_{16}H_{23}ON$: C, 78·3; H, 9·4%).

(*l*) NN-*Di*-n-*propyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Reaction of the above amide (3·02 g; 0·012 mole) with lithium aluminium hydride (0·7 g; 0·018 mole) in ether (40 ml) by the method

described in (*d*) for 8 hr gave the amine (2·32 g, 81% yield) as a colourless oil, b.p. 124–126 °C/0·8 mm, n_D^{17} 1·5180.

The *hydrochloride* crystallized from ethanol as needles, m.p. 154–155 °C (Found: C, 71·6; H, 9·6%. Calc. for $C_{16}H_{26}NCl$: C, 71·8; H, 9·8%).

(*m*) N-*Butyryl-1,2,3,4-tetrahydro-2-naphthylamine*.—Reaction of 1,2,3,4-tetrahydro-2-naphthylamine (7·5 g; 0·05 mole) with butyryl chloride (6 g; 0·056 mole) by the method described in (*h*) for 1 hr afforded the *amide* as white feathery needles from light petroleum, m.p. 84 °C (9·1 g, 84% yield) (Found: C, 77·2; H, 8·8%. Calc. for $C_{14}H_{19}ON$: C, 77·4; H, 8·8%).

(*n*) N-n-*Butyl-1,2,3,4-tetrahydro-2-naphthylamine*.—The above amide (9 g; 0·041 mole) was reduced with lithium aluminium hydride (2·5 g; 0·063 mole) as described in (*d*) for 8 hr, affording the amine (6·78 g, 81% yield) as a colourless oil, b.p. 138–140 °C/2·8 mm, n_D^{22} 1·5255.

The *hydrochloride* formed needles from ethanol-ether, m.p. 217 °C (Found: C, 70·0; H, 9·3%. Calc. for $C_{14}H_{22}NCl$: C, 70·1; H, 9·3%).

(*o*) N-n-*Butyl*-N-*methyl-1,2,3,4-tetrahydro-2-naphthylamine*.—Treatment of the above amine (2·86 g; 0·014 mole) with formic acid (4 ml) and formalin (2 g of 40%; 0·027 mole) by the method used in (*a*) for 3 hr afforded the tertiary amine (2·13 g, 70% yield) as a colourless oil, b.p. 115–117 °C/1 mm, n_D^{22} 1·5222.

The *hydrobromide* formed needles from ethanol, m.p. 143–145 °C (Found: C, 60·0; H, 7·9%. Calc. for $C_{15}H_{24}NBr$: C, 60·4; H, 8·1%).

(*p*) N-iso-*Butyl-1,2,3,4-tetrahydro-2-naphthylamine*.—A mixture of 1,2,3,4-tetrahydro-2-naphthylamine (12·2 g; 0·08 mole) and *iso*butyraldehyde (12 g; 0·04 mole) was refluxed for 30 min and excess aldehyde removed by distillation. The residue gave 16 g (96% yield) of the Schiff's base, b.p. 104–106 °C/0·9 mm, n_D^{21} 1·5231.

The above substance (14·73 g; 0·07 mole) in ethyl acetate (500 ml) was shaken with hydrogen and Adams's catalyst (0·1 g) until 1 mol of hydrogen had been absorbed (4 hr). Removal of catalyst and distillation gave the amine (13 g, 88% yield) as a pale yellow oil, b.p. 110–112 °C/1 mm, n_D^{22} 1·5200.

The *hydrochloride* crystallized as plates from ethanol-ethyl acetate, m.p. 254–255 °C (Found: C, 69·8; H, 9·0%. Calc. for $C_{14}H_{22}NCl$: C, 70·1; H, 9·2%).

(*q*) N-iso-*Butyl*-N-*methyl-1,2,3,4-tetrahydro-2-naphthylamine*.—The above amine (9·7 g), formic acid (10 ml), and formalin (8 g of 40% solution) were reacted as described in (*a*), giving the tertiary amine (6·3 g, 61% yield) as an oil, b.p. 98–100 °C/0·7 mm, n_D^{16} 1·5200.

The *hydriodide* formed needles from ethanol-ether, m.p. 197–198 °C (Found: C, 52·0; H, 7·0%. Calc. for $C_{15}H_{24}NI$: C, 52·2; H, 7·0%).

III. Acknowledgments

The authors are indebted to Professor Thorp and Miss J. N. Pennefather for examination of the compounds, and to the University of Sydney for the award of a Scholarship (B.M.).

IV. References

Bamberger, E., and Müller, R. (1889).—*Ber. dtsch. chem. Ges.* **22**: 1295.
Bovet, D., Sollero, L., and Marotta, M. (1952).—*R.C. Ist. Sup. Sanit.* **15**: 905.
Bovet, D., and Virno, M. (1952).—*R.C. Ist. Sup. Sanit.* **15**: 870.
Brown, D. L., and Dale, H. H. (1935).—*Proc. Roy. Soc.* B **118**: 446.
Chiavarelli, S., Vittory, R. L., Marzadro, M., and Palazzo, G. (1952).—*R.C. Ist. Sup. Sanit.* **15**: 862.
Cloetta, M., and Waser, E. (1913).—*Arch. exp. Path. Pharmak.* **73**: 398.
Gaddum, J. H. (1953).—*J. Physiol.* **121**: 15.
Jacobs, W., and Craig, L. C. (1934*a*).—*J. Biol. Chem.* **104**: 547.
Jacobs, W., and Craig, L. C. (1934*b*).—*J. Biol. Chem.* **106**: 393.

Kornfeld, E. C., Fornefeld, E. J., Kline, G. B., Mann, M. J., Morrison, D. E., Jones, R. G., and Woodward, R. B. (1956).—*J. Amer. Chem. Soc.* **78** : 3087.
Kraushaar, A. (1954).—*Arzniemittel-Forsch.* **4** : 273.
Marini-Bettolo, G. B., Chiavarelli, S., and Bovet, D. (1950).—*Gazz. chim. ital.* **80** : 281.
Marini-Bettolo, G. B., Chiavarelli, S., and Bovet, D. (1952).—*R.C. Ist. Sup. Sanit.* **15** : 767.
Marini-Bettolo, G. B., Vittory, R. L., and Bovet, D. (1952).—*R.C. Ist. Sup. Sanit.* **15** : 844.
Pennefather, J. N., and Thorp, R. H. (1958).—*J. Pharm. Pharmacol.* **10** : 249.
Pennefather, J. N., and Thorp, R. H. (1959).—*Arch. Int. Pharmacodyn.* (in press).
Rothlin, E. (1923).—*Arch. Inst. Pharmacodyn.* **27** : 459.
Rothlin, E. (1947).—*Bull. schweiz. Akad. med. Wiss.* **2** : 249.
Rothlin, E. (1957).—*J. Pharm. Pharmacol.* **9** : 569.
Smith, S., and Timmis, G. M. (1932*a*).—*J. Chem. Soc.* **1932** : 763.
Smith, S., and Timmis, G. M. (1932*b*).—*J. Chem. Soc.* **1932** : 1543.
Stoll, W. A. (1947).—*Schweiz. Arch. Neurol. Psychiat.* **60** : 279.
Stoll, W. A., Hofmann, A., and Troxler, F. (1949).—*Helv. Chim. Acta* **32** : 506.
Waser, E. (1916).—*Ber. dtsch. chem. Ges.* **49** : 1202.

SIMPLIFIED ANALOGUES OF LYSERGIC ACID

II. DERIVATIVES OF 3-(*o*-TOLYL)-*n*-PROPYLAMINE AND 1-(*o*-TOLYL)*iso*PROPYLAMINE

By J. CYMERMAN CRAIG,* B. MOORE,* and E. RITCHIE*

[*Manuscript received April* 22, 1959]

Summary

Several *N*-alkyl derivatives of 3-(*o*-tolyl)-*n*-propylamine and 1-(*o*-tolyl)*iso*propylamine have been prepared and examined for lysergic acid-like activity.

I. INTRODUCTION

Because of the interesting pharmacological activity of derivatives of 1,2,3,4-tetrahydro-2-naphthylamine (I) (Cymerman-Craig, Moore, and Ritchie 1959), particularly the high and specific antiserotonin activity of *N*-methyl-*N*-*n*-propyl-1,2,3,4-tetrahydro-2-naphthylamine (Pennefather and Thorp 1958), the possibility existed that activity might be retained even after opening of ring C as well as D and loss of ring B in the lysergic acid molecule (II).

Of the several ways in which ring C may be envisaged as opening, one leads to 3-(*o*-tolyl)-*n*-propylamine (III, $R_1=R_2=H$) and the other to 1-(*o*-tolyl)*iso*-propylamine (IV, $R_1=R_2=H$), and both may therefore be regarded as open-chain analogues of I.

A series of *N*-(β-phenylethyl)-*N*-methyl-β-alanine esters (V), which may be considered as analogues of II, was found by Baltzly, Dvorkovitz, and Phillips (1949) to have about 5 to 10 per cent. of the oxytocic activity of ergonovine when the aromatic ring contained two alkoxy substituents; the unsubstituted compounds were inactive, and monoalkoxy substitution gave only one-tenth of the activity of dialkoxy compounds.

The closely related *N*-(β-hydroxy-β-phenylethyl)-*N*-methyl-β-alanine esters (VI) (Baltzly and Phillips 1949*a*) had the same order of oxytocic activity as the compounds of type V, while examination of substances of general type VII (Baltzly and Phillips 1949*b*) indicated that only those in which $m=2$ and where $n\neq 2$, were active.

Plieninger (1953) observed oxytocic activity in the open-chain compound VIII, and found that while the related 5-(*p*-methoxyphenyl)-6-methylpiperidine-3-carboxylic acid esters of type IX showed only weak action on the rabbit uterus, the corresponding unsaturated systems X had markedly higher potency.

In a series of 3-(2[1]-piperidyl)methylindoles (XI) and 3-(*N*-piperidyl)methyl-indoles (XII) synthesized by Akkerman, de Jongh, and Veldstra (1951) and Akkerman and Veldstra (1954), the highest activity was attained in compounds of type XII; for example, XII ($R_1=R_2=Me$; $R_3=H$) had activity equal to

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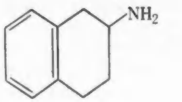
half of ergometrine by weight. The presence of an ester or amide group in the piperidine ring was dystherapeutic, and compounds of type XI, though closer chemically to II, were markedly less active. Moreover, the work of Schindler



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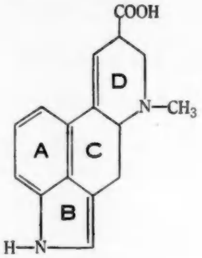

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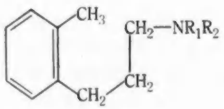

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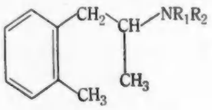

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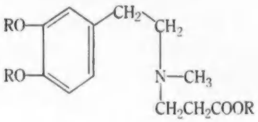
and Voegtli (1949) showed that simple compounds such as *N*-piperidylmethyl-*cyclo*pentane and -hexane had about one-tenth of the activity of methyl ergobasine on the guinea pig uterus.



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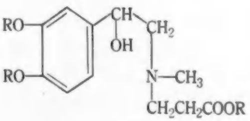

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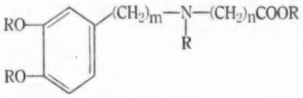

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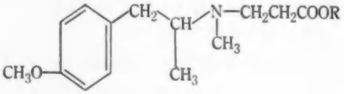

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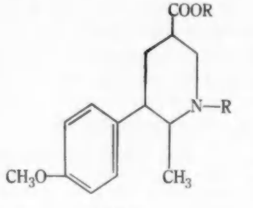
The preparation of substances of types III and IV was accordingly undertaken, *o*-tolualdehyde serving as the starting material for both series. For type III, it was first condensed with malonic acid in the presence of pyridine and piperidine to form *o*-methylcinnamic acid, which was reduced in alkaline solution by Raney nickel alloy, or better by hydrogen with platinum, to *o*-methyldihydrocinnamic acid. This was converted to the acid chloride, which on reaction

with ammonia, methylamine, dimethylamine, ethylamine, diethylamine, *n*-propylamine, and *n*-butylamine, afforded the respective amides. Reduction with lithium aluminium hydride then gave 3-(*o*-tolyl)-*n*-propylamine (III, $R_1=R_2=H$) and the corresponding *N*-alkylated derivatives. Also, because of the activity of *N*-methyl-*N*-*n*-propyl-1,2,3,4-tetrahydro-2-naphthylamine (Pennefather and Thorp 1958), methylation of (III, $R_1=H$, $R_2=n\text{-}C_3H_7$) was effected by formaldehyde-formic acid.



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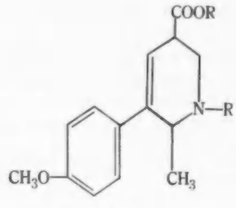

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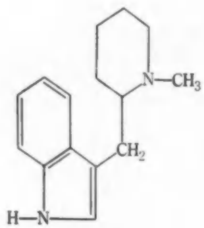

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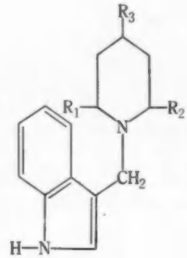

(XII)

In the other series, *o*-tolualdehyde was condensed with nitroethane by the method of Burger and Foggio (1956) to 1-(*o*-tolyl)-2-nitropropene-1, which was reduced by lithium aluminium hydride to 1-(*o*-tolyl)*iso*propylamine (IV, $R_1=R_2=H$).

Further members of the series were not prepared because of the report by Miss J. N. Pennefather and Professor R. H. Thorp of the Pharmacology Department of the University of Sydney, that none of these compounds showed interesting pharmacological activity. It thus appears that in the lysergic acid analogues devoid of ring B, the opening of ring C as well as ring D results in the loss of activity in the residual fragments III and IV.

II. Experimental

Melting points are uncorrected. The analyses were carried out by Dr. K. W. Zimmermann, C.S.I.R.O. and University of Melbourne Microanalytical Laboratory, and by Miss B. Stevenson, University of Sydney. Light petroleum refers to the fraction of b.p. 60–90 °C.

(*a*) o-*Methylcinnamic Acid*.—A mixture of *o*-tolualdehyde (32 g), malonic acid (40 g), dry pyridine (150 ml), and dry piperidine (20 ml) was heated under reflux on the steam-bath for 4 hr,

cooled, and then poured into concentrated hydrochloric acid (250 ml) and crushed ice (500 g). The product was collected, washed well with water, dried, and recrystallized from benzene as lustrous cream plates (32 g, 75% yield), m.p. 175–176 °C (lit. m.p. 174–175 °C).

(*b*) o-*Methyldihydrocinnamic Acid*.—A solution of *o*-methylcinnamic acid (33·5 g) in hot absolute ethanol (600 ml) was hydrogenated with the aid of platinum oxide (0·5 g). When uptake of hydrogen ceased after about 3 hr, the catalyst was filtered off and the filtrate concentrated to a small bulk. On cooling, the product separated as colourless needles (31·1 g), m.p. 104–105 °C (lit. m.p. 102 °C).

(*c*) *3-*(o-*Tolyl*)*propionyl Chloride*.—A mixture of the acid (31 g), dry benzene (50 ml), and thionyl chloride (30 g) was refluxed for 0·5 hr and then the solvent and excess reagent removed. The residue on distillation afforded a colourless oil (yield 95%), b.p. 81–82 °C/0·7 mm.

(*d*) *3-*(o-*Tolyl*)*propionamide*.—The acid chloride (4 g) was added slowly with stirring and cooling to excess concentrated ammonia. The resulting pasty mixture was diluted, the *product* collected and recrystallized from benzene yielding colourless needles (2·8 g), m.p. 113–114 °C (Found: C, 73·6; H, 7·9%. Calc. for $C_{10}H_{13}ON$: C, 73·6; H, 8·0%).

(*e*) *3-*(o-*Tolyl*)-n-*propylamine*.—The amide (2·2 g) was reduced with lithium aluminium hydride (0·75 g) in dry ether (80 ml) by heating under reflux for 4 hr. Water was cautiously added, the ethereal layer separated and combined with an ether extract of the aqueous layer. The base was extracted from the ether solution with dilute acid, regenerated, and eventually isolated by distillation. It (1·6 g) formed a colourless oil, b.p. 100–101 °C/2·7 mm, n_D^{22} 1·5270.

The *hydrochloride*, prepared in dry ether with dry hydrogen chloride, crystallized from absolute ethanol–ethyl acetate in lustrous plates, m.p. 186 °C (Found: C, 64·5; H, 8·5%. Calc. for $C_{10}H_{16}NCl$: C, 64·7; H, 8·7%).

(*f*) N-*Methyl-3-*(o-*tolyl*)*propionamide*.—Dry methylamine in excess was passed into a solution of the acid chloride (3 g) in dry benzene (50 ml). After washing with dilute acid and water, the benzene solution was dried and evaporated. The residue on distillation yielded a colourless *gum* (1·9 g), b.p. 159–160 °C/1·2 mm (Found: C, 74·4; H, 8·6%. Calc. for $C_{11}H_{15}ON$: C, 74·5; H, 8·5%).

(*g*) N-*Methyl-3-*(o-*tolyl*)-n-*propylamine*.—Reduction of the amide (1·7 g) with lithium aluminium hydride (0·6 g) as usual, gave a colourless oil (1·33 g), b.p. 82–83 °C/0·9 mm, $n_D^{22\cdot5}$ 1·5129.

The *hydrochloride* crystallized from absolute ethanol–ether in colourless needles, m.p. 145 °C (Found: C, 65·9; H, 9·1%. Calc. for $C_{11}H_{18}NCl$: C, 66·1; H, 9·1%).

(*h*) NN-*Dimethyl-3-*(o-*tolyl*)-n-*propylamine*.—The dimethylamide prepared as in (*f*) was a thick oil, b.p. 152–154 °C/3·5 mm, n_D^{23} 1·5350. Reduction as usual gave the amine (70% yield), b.p. 76–77 °C/0·7 mm, n_D^{24} 1·5030.

The *hydrochloride* separated from absolute ethanol–ethyl acetate as colourless plates, m.p. 166 °C (Found: C, 67·1; H, 9·4%. Calc. for $C_{12}H_{20}NCl$: C, 67·4; H, 9·4%).

(*i*) N-*Ethyl-3-*(o-*tolyl*)-n-*propylamine*.—The *ethylamide*, prepared as usual, crystallized from light petroleum in colourless needles, m.p. 60 °C (Found: C, 75·4; H, 8·5%. Calc. for $C_{12}H_{17}ON$: C, 75·4; H, 9·0%).

By reduction as usual, the amine was obtained as a colourless oil (70% yield), b.p. 90–92 °C/0·9 mm, n_D^{21} 1·5882.

The *hydrochloride* crystallized from absolute ethanol–ethyl acetate in plates, m.p. 140 °C (Found: C, 67·2; H, 9·4%. Calc. for $C_{12}H_{20}NCl$: C, 67·4; H, 9·4%).

(*j*) NN-*Diethyl-3-*(o-*tolyl*)-n-*propylamine*.—The *diethylamide* was a thick oil, b.p. 132–134 °C/1 mm, n_D^{23} 1·5232 (Found: C, 76·4; H, 9·2%. Calc. for $C_{14}H_{21}ON$: C, 76·7; H, 9·7%).

Lithium aluminium hydride reduction gave the base (85% yield), b.p. 93–94 °C/0·9 mm, $n_D^{21\cdot5}$ 1·5071.

The *hydriodide* crystallized from absolute ethanol–ether in colourless needles, m.p. 109 °C (Found: C, 50·7; H, 7·3%. Calc. for $C_{14}H_{24}NI$: C, 50·4; H, 7·3%).

(*k*) N-n-*Propyl-3-*(o-*tolyl*)-n-*propylamine*.—The n-*propylamide* had b.p. 172–174 °C/2 mm, n_D^{21} 1·5257 (Found: C, 75·8; H, 9·1%. Calc. for $C_{13}H_{19}ON$: C, 76·1; H, 9·3%).

The base (80% yield) obtained by reduction was an oil, b.p. 98 °C/0·7 mm, n_D^{22} 1·5041.

The *hydrochloride* separated from absolute ethanol–ethyl acetate in needles, m.p. 177–179 °C (Found: C, 68·6; H, 9·8%. Calc. for $C_{13}H_{22}NCl$: C, 68·5; H, 9·7%).

(*l*) N-*Methyl*-N-n-*propyl-3-*(o-*tolyl*)-n-*propylamine*.—Methylation of the above amine (2·0 g) with formaldehyde (3 ml; 40%) and formic acid (3 ml) under reflux for 3 hr in the usual manner, gave the *product*, b.p. 96–98 °C/1 mm, n_D^{16} 1·5020 (Found: C, 81·4; H, 11·0%. Calc. for $C_{14}H_{23}N$: C, 81·9; H, 11·3%).

(*m*) N-n-*Butyl-3-*(o-*tolyl*)-n-*propylamine*.—The usual procedure afforded the *amide* as colourless needles, m.p. 57 °C, from light petroleum (Found: C, 76·8; H, 9·8%. Calc. for $C_{14}H_{21}ON$: C, 76·7; H, 9·7%).

Reduction gave the base (75% yield), b.p. 130–132 °C/2·8 mm, $n_D^{22\cdot5}$ 1·5012.

The *hydrochloride* crystallized from absolute ethanol–ethyl acetate in needles, m.p. 195 °C (Found: C, 69·3; H, 10·1%. Calc. for $C_{14}H_{24}NCl$: C, 69·5; H 10·0%).

(*n*) *1-*(o-*Tolyl*)*isopropylamine*.—A solution of *o*-tolualdehyde (35 g), nitroethane (33·5 g), and ammonium acetate (16·5 g) in glacial acetic acid (450 ml) was refluxed for 3 hr, then cooled, and diluted with an equal volume of ice-water. The mixture was extracted with ether and the ethereal solution washed in turn with water, aqueous sodium carbonate, and water, dried and distilled. The nitropropene (29 g) was a pale yellow oil, b.p. 122–124 °C/4 mm, n_D^{19} 1·5800.

A solution of the nitropropene (29 g) in dry ether (400 ml) was added slowly to a suspension of lithium aluminium hydride (15·5 g) in dry ether (200 ml). When the extremely vigorous reaction had subsided, the mixture was refluxed for 10 hr and then decomposed by the careful addition of ice-water. The ethereal layer was separated and the residue subjected to continuous ether extraction for 5 hr. The combined ether extracts were shaken with 10% hydrochloric acid and the *product* isolated in the usual manner. It was a colourless oil, b.p. 58–60 °C/0·9 mm, n_D^{22} 1·5264 (Found: C, 80·0; H, 9·9%. Calc. for $C_{10}H_{15}N$: C, 80·5; H, 10·1%).

III. Acknowledgment

The authors are indebted to the University of Sydney for the award of a scholarship to one of them (B.M.).

IV. References

Akkermann, A. M., de Jongh, D. K., and Veldstra, H. (1951).—*Rec. Trav. chim. Pays-Bas* **70**: 899.

Akkermann, A. M., and Veldstra, H. (1954).—*Rec. Trav. chim. Pays-Bas* **73**: 629.

Baltzly, R., Dvorkovitz, V., and Phillips, A. P. (1949).—*J. Amer. Chem. Soc.* **71**: 1162.

Baltzly, R., and Phillips, A. P. (1949*a*).—*J. Amer. Chem. Soc.* **71**: 3149.

Baltzly, R., and Phillips, A. P. (1949*b*).—*J. Amer. Chem. Soc.* **71**: 3421.

Burger, A., and Foggio, R. D. (1956).—*J. Amer. Chem. Soc.* **78**: 4419.

Cymerman Craig, J., Moore, B., and Ritchie, E. (1959).—*Aust. J. Chem.* **12**: 447.

Pennefather, J. N., and Thorp, R. H. (1958).—*J. Pharm. Pharmacol.* **10**: 249.

Plieninger, H. (1953).—*Chem. Ber.* **86**: 25.

Schindler, O., and Voegtli, W. (1949).—*Pharm. Acta Helv.* **24**: 207.

ALKALOIDS OF THE AUSTRALIAN RUTACEAE: *LUNASIA QUERCIFOLIA*

II. THE NATURE OF LUNASINE

By J. R. Price*

[*Manuscript received April* 16, 1959]

Summary

The principal component of the mixture of water-soluble alkaloids of *Lunasia quercifolia* is shown to be the quaternary 4-methoxy-1-methylquinolinium structure IV corresponding to the 1-methyl-4-quinolone alkaloid lunacrine. The formation of IV from lunacrine and methyl iodide involves addition of the methyl group to the quinolone oxygen atom. It is suggested that this, as with the γ-pyrones, is the usual mode of reaction of 4-quinolone with alkyl halides. The properties of 4-methoxyquinolinium salts are discussed in relation to the conversion of furoquinoline alkaloids to the *iso*-alkaloids.

I. Introduction†

Alkaloids from the genus *Lunasia* have been examined by a number of workers, but no detailed structural studies had been made until recently, when 4-methoxy-2-phenylquinoline (Goodwin, Smith, and Horning 1957) and 7-methoxy-1-methyl-2-phenyl-4-quinolone (Price 1956; Johnstone, Price, and Todd 1958) were identified. The former was isolated from leaves of *L. amara* and the latter, together with the previously described lunacrine and lunine, from the bark of *L. quercifolia*. Johnstone, Price, and Todd also reported the presence of large amounts of alkali-sensitive water-soluble bases in the bark and leaves of both species, at least four components being revealed by paper chromatography. The work reported here is concerned with the identification of the major component of the "water-soluble base" fraction from *L. quercifolia* bark. The water-soluble bases are not *N*-oxides, since they are not reduced by zinc and sulphuric acid. They may be precipitated, after removal of the tertiary bases, as picrates, perchlorates, or other salts. Repeated crystallization of the mixed picrates gives the laevorotatory picrate, $C_{17}H_{22}O_3N^+.C_6H_2O_7N_3^-$. This picrate and other $C_{17}H_{22}O_3N^+$ salts are converted by hydroxyl ion into a dextrorotatory, weakly basic substance $C_{17}H_{23}O_4N$, the properties of which clearly show it to be identical with the alkaloid *lunacridine* first described by Boorsma (1904) and subsequently by Steldt and Chen (1943).‡ In addition to lunacridine and lunacrine, Boorsma isolated a water-soluble alkaloid which he named lunasine§

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† A preliminary account has been given by Price (1958).

‡ Unfortunately the name lunacridine was also applied by Dieterle and Beyl (1937) to a base $C_{16}H_{15}O_4N$, which melted almost 150 °C higher than the melting point of Boorsma's lunacridine.

§ This name was originally given to a water-soluble alkaloid from *Lunasia amara* bark by Lewin (1897), but the plant material was of doubtful authenticity.

and which he showed to be converted into lunacridine by alkalis. Boorsma's " lunasine " is therefore closely related to the salts described in the present work and, as will be evident, must have been a salt or mixture of salts.*

Analysis of the picrate $C_{17}H_{22}O_3N^+.C_6H_2O_7N_3^-$, which, to maintain continuity with the earlier work, will be termed lunasine picrate, shows the presence of two methoxyl groups and one methylimino group ; the *C*-methyl result was somewhat less than is required for one carbon-methyl group. Lunacridine likewise contains two methoxyl groups and one methylimino group, and again

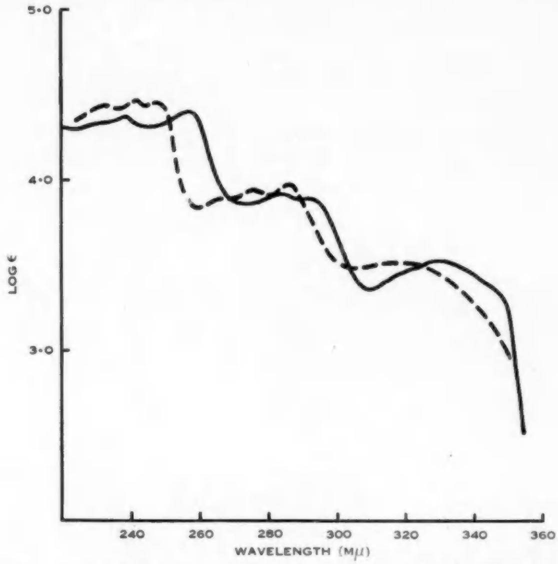


Fig. 1.—Ultraviolet spectrum of —— lunacridine in ethanol; — — — 4-hydroxy-8-methoxy-2-quinolone (redrawn on the logarithmic scale from the data of Deulofeu and Bassi (1952)).

the result of the *C*-methyl analysis was somewhat below 90 per cent. of the calculated requirement for one *C*-methyl group, a figure consistent with either one *C*-methyl or a *gem*-dimethyl grouping. The very low basicity leaves little doubt that lunacridine is either an amide or a 2-quinolone ; the ultraviolet absorption spectrum is characteristic of the latter and closely resembles that of 4-hydroxy-8-methoxy-2-quinolone (Fig. 1) reported by Deulofeu and Bassi (1952). The infra-red spectrum is also consistent with a quinolone structure ; the carbonyl frequency at 1633 cm^{-1} is a little high for a 4-quinolone and somewhat lower than usually shown by 2-quinolones, though Grundon and

* The name lunasine, designating a water-soluble base from *L. amara*, has been used by at least six groups of investigators. The procedures employed by Boorsma (1904), Wirth (1931), and Dieterle and Beyl (1937) in the isolation of " lunasine " do not exclude the possibility that the product was in each instance a salt or mixture of salts.

McCorkindale (1957) cite an instance of a 2-quinolone which absorbs at 1631 cm^{-1} (see also Price and Willis 1959). Lunacridine is not soluble in alkali, but contains an hydroxyl group as shown by infra-red absorption at 3370 cm^{-1} and by the formation of a monoacetyl derivative. Vigorous treatment with acid converts lunacridine in part into a more basic substance $C_{16}H_{19}O_3N$, m.p. 117–118 °C, identical with the alkaloid lunacrine in infra-red spectrum and other physical properties, but differing in optical rotation. Lunacrine isolated from *Lunasia* sp.

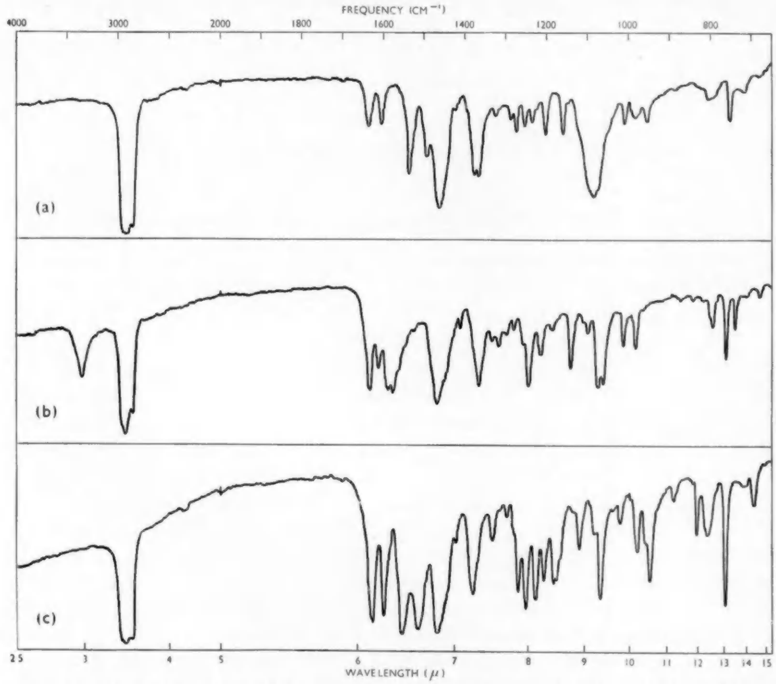
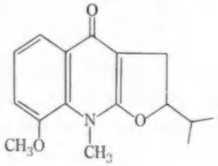


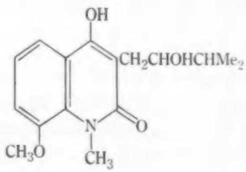
Fig. 2.—Infra-red spectra (Nujol mulls) of (*a*) lunasine perchlorate ; (*b*) lunacridine ; (*c*) lunacrine.

has $[\alpha]_D$ $-51°$; the compound from (+)-lunacridine $[\alpha]_D$ $+50°$; a mixture of the two melts at 145–146 °C and a small elevation of melting point was observed with a mixture of the picrates. Dieterle and Beyl (1937) report the conversion of (−)-lunacrine—either by the action of dimethyl sulphate followed by alkali or by the action of alkali on lunacrine methiodide—into a substance of m.p. 85 °C. As this is the melting point of lunacridine, these reactions were repeated. Identification of the product with the (+)-lunacridine obtained from lunasine picrate suggested that lunasine salts may be identical with the corresponding methyl lunacrinium salts and this was established by direct comparison. It was found subsequently that (−)-lunasine picrate can be converted directly to (−)-lunacrine picrate by heating with acid.

While this work was in progress the structure of lunacrine was under investigation by Dr. S. M. Goodwin and Dr. E. C. Horning of the National Heart Institute, National Institutes of Health, Bethesda, U.S.A., who very kindly exchanged information and materials relating to the problem. Goodwin and Horning (1959) and Goodwin, Shoolery, and Johnson (1959) have now established that lunacrine is the *iso*propyldihydrofuroquinolone (I), the position of the methoxyl group being confirmed and the nature of the side chain established by nuclear magnetic resonance spectral analysis. Lunacrine, as found in both laboratories, is converted by the action of ethanolic potassium hydroxide to a laevorotatory phenol, $C_{16}H_{21}O_4N$. The infra-red absorption spectrum of this substance in the carbonyl region (1638 cm^{-1}) and its sparing solubility in acid imply that it is a 2-quinolone and, indeed, the structure II follows from the method of preparation from I. Likewise, by its formation on methylating II with diazomethane, Goodwin and Horning showed lunacridine to be the 4-methoxy-2-quinolone (III). It follows that the methyl lunacrinium ion,



(I)



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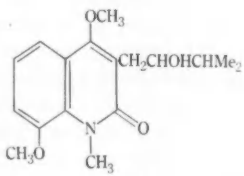

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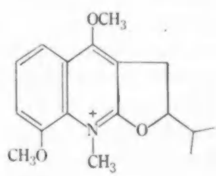
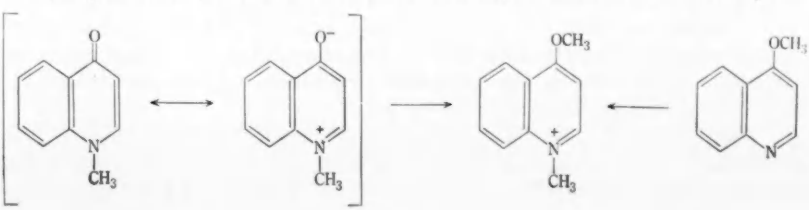


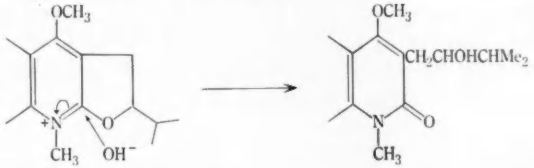
(IV)

from which the lunasine salts derive, has structure IV. The formation of the 4-methoxyquinolinium compound (IV) from I and methyl iodide resembles the reaction of the γ-pyrones with alkyl halides. As this resemblance is also found with 1-methyl-4-quinolone, the " methiodide " of which has now been shown to be identical with that prepared from 4-methoxyquinoline, it may be inferred that, in general, 4-quinolones which react with alkyl halides do so in this way :



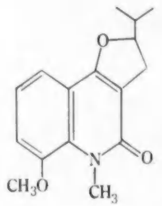
For example, Adams and Hey (1951) describe the methiodides of 4-methoxy-3-phenylquinoline and 1-methyl-3-phenyl-4-quinolone. Both methiodides decomposed on attempted crystallization and neither, therefore, could be purified; the former melted at 151–153 °C, the latter at 156·5–161·5 °C. It is suggested that both preparations were 4-methoxy-1-methyl-3-phenylquinolinium iodide.

Goodwin and Horning have discussed mechanisms for the alkaline hydrolysis of lunacrine and the cyclization of lunacridine. They regard the hydrolysis as proceeding without inversion and the cyclization with inversion. This interpretation is consistent with the formation, without inversion, of (+)-lunacridine from the methyl (−)-lunacrinium ion:



This transformation proceeds with great facility; when an aqueous solution of methyl lunacrinium (lunasine) iodide is made 0·25N with respect to sodium hydroxide at room temperature the mixture becomes opalescent in a few seconds and lunacridine crystallizes. The methiodide of 4-methoxyquinoline behaves likewise, being converted quantitatively to 1-methyl-4-quinolone, while under the same conditions 7-methoxy-1-methyl-2-phenyl-4-quinolone is regenerated from its " methiodide ", 4,7-dimethoxy-2-phenylquinolinium iodide.

The acid-catalysed cyclization of (+)-lunacridine to (+)-lunacrine gives rise to a mixture of products of which the (+)-lunacrine represents *c.* 30 per cent. The major product is isomeric with lunacrine, laevorotatory, absorbs at 1666 cm^{-1},



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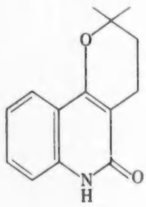


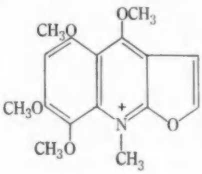
(VI)

and resembles lunacridine in being very weakly basic. It is clearly the angular 2-quinolone (V). Unlike lunacrine, V is not attacked by boiling ethanolic potassium hydroxide, resembling in this respect the dihydro derivative (VI) of the alkaloid flindersine (Brown *et al.* 1954).

The phenol (II), like lunacridine, also undergoes cyclization by acid to a mixture of lunacrine and the angular compound V, the yield of lunacrine being less from II, as might be expected if angular cyclization of lunacridine involves

initial demethylation. However, the cyclization is not so straightforward in this instance, since the lunacrine though largely (+)-isomer is not optically pure, racemization having taken place to a small extent.

The structure and properties of the 4-methoxyquinolinium salts obtained from 4-quinolones are of interest in connection with the conversion of 4-alkoxyquinolines to *N*-alkyl quinolones. For example, the well-known conversion of the furoquinoline alkaloids—4-methoxy compounds—to the *iso*alkaloids—1-methyl-4-quinolones—by the action of methyl iodide might, by analogy with lunacrine, have been expected to give rise to a quaternary quinolinium iodide analogous to the methyl lunacrinium salts. However, no methiodide of a furoquinoline appears to have been described (see Openshaw 1953). Dictamnine is stated to be unaffected by boiling methyl iodide, but is converted to *iso*-



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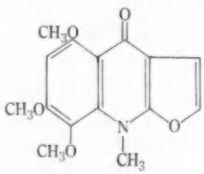

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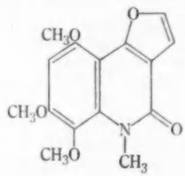

(IX)

dictamnine when heated with the halide in a sealed tube at 80 °C. Probably because of this behaviour, subsequent workers, with other furoquinoline alkaloids, have generally prepared the *iso*alkaloid by heating with methyl iodide in a sealed tube. It is now found that acronycidine reacts slowly with methyl iodide at room temperature, giving a water-soluble quaternary iodide (VII) which decomposes easily to *iso*acronycidine (VIII) either on heating or on treatment with hydroxyl ion. However, VII could not be prepared from *iso*acronycidine. The formation from acronycidine of the angular isomer IX, described by Lahey, Lamberton, and Price (1950) though it takes place under alkaline conditions resembles the conversion of lunacrine to lunacridine via the quaternary ion IV, followed by recyclization to an analogue of the angular compound V formed from lunacridine.

II. Experimental

Microanalyses were made by the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory. All melting points are corrected unless otherwise stated. Infra-red spectra were measured on a Perkin-Elmer Model 21 Spectrophotometer ; ultraviolet spectra with a Hilger Uvispek Spectrophotometer.

(a) *Isolation of Salts of (—)-Lunasine*

The aqueous acidic solution of mixed water-soluble bases after extraction of the tertiary bases (Johnstone, Price, and Todd 1958) was extracted with phenol, the phenol solution washed twice with a little water, diluted with several volumes of ether, and shaken with a little water. To the resulting concentrated aqueous solution of water-soluble bases, a portion of which gave rise to only a negligible quantity of chloroform-extractable tertiary bases after reduction with zinc and sulphuric acid, was added a saturated aqueous solution of picric acid until there was no further separation of picrate. Repeated crystallization of the mixed picrates from methanol gave

(—)-lunasine picrate, yellow needles, m.p. 143·5–144 °C (Found: C, 53·6; H, 4·8; N, 10·8; CH_3O, 11·8; $CH_3(N)$, 3·4; $CH_3(C)$, 2·6%; $[\alpha]_D^{20}$ —20·3° (*c*, 0·98 in methanol). Calc. for $C_{17}H_{22}O_3N^+.C_6H_2O_7N_3^-$: C, 53·5; H, 4·7; N, 10·9; 2×CH_3O, 12·0; $CH_3(N)$, 2·9; $CH_3(C)$, 2·9%). A chromatogram run with water-saturated *iso*pentanol on paper impregnated with 1 : 1 pH 2·2 McElvain buffer : glycol showed only one spot R_F 0·7, fluorescing light blue under ultraviolet light, and corresponding to the major spot on chromatograms of the mixed water-soluble bases.

(—)-Lunasine picrate (500 mg) was refluxed with hydrochloric acid (5N; 100 ml) for 80 hr, the solution was then concentrated to *c*. 30 ml and diluted with water. The resulting yellow precipitate (160 mg) after crystallization first from aqueous methanol and finally from acetone gave (—)-lunacrine picrate, m.p. 206·5–208 °C, undepressed on admixture with an authentic specimen.

(—)-Lunasine perchlorate was prepared by dissolving the picrate in boiling methanol, adding a small excess of perchloric acid, and diluting with ether. Repeated crystallization from methanol–ether gave a colourless microcrystalline powder, m.p. 195–196 °C (Found: C, 52·9; H, 5·9; O, 28·5; N, 3·4%; $[\alpha]_D^{20}$ —29·3° (*c*, 0·96 in methanol). Calc. for $C_{17}H_{22}O_3N^+.ClO_4^-$: C, 52·6; H, 5·7; O, 28·9; N, 3·4%).

(*b*) *Preparation of* (—)-*Lunasine Salts from* (—)-*Lunacrine*

(—)-Lunacrine methiodide crystallized from a solution of lunacrine in methyl iodide which was allowed to stand at room temperature for 48 hr. Recrystallized from ethanol, it melted at 129·5 °C (decomp.) and had $[\alpha]_D^{20}$ —27·9° (*c*, 1·29 in ethanol). Dieterle and Beyl (1937) report m.p. 130–131 °C, R_F (on paper impregnated with 1 : 1 pH 2·2 McElvain buffer : glycol; mobile phase, water-saturated *iso*pentanol), 0·7. (—)-Lunacrine methopicrate prepared from (—)-lunacrine methiodide, crystallized from ethanol as yellow needles, m.p. and mixed m.p. with (—)-lunasine picrate 144–145 °C. (—)-Lunacrine methoperchlorate prepared from the methiodide crystallized from methanol–ether as small colourless prisms, m.p. and mixed m.p. with lunasine perchlorate 193–194 °C.

(*c*) (+)-*Lunacridine from* (—)-*Lunasine Salts*

(—)-Lunasine picrate (2·0 g) was dissolved in warm methanol (50 ml), aqueous sodium hydroxide (5%; 10 ml) added, and the methanol removed under reduced pressure on the water-bath. The mixture was diluted with water, extracted with chloroform, and the chloroform evaporated leaving (+)-lunacridine (1·17 g, 99%). Crystallization from aqueous methanol, or from light petroleum gave (+)-lunacridine as colourless needles, m.p. 85–86 °C (Found: C, 67·1; H, 7·5; N, 4·6; CH_3O, 19·7; $CH_3(N)$, 6·5; $CH_3(C)$, 4·2%; $[\alpha]_D^{20}$ +29·2° (*c*, 1·79 in ethanol). Calc. for $C_{17}H_{23}O_4N$: C, 66·9; H, 7·5; N, 4·6; 2×CH_3O, 20·1; $CH_3(N)$, 4·9; $CH_3(C)$, 4·9%). (+)-Lunacridine is insoluble in aqueous sodium hydroxide and in N hydrochloric acid but dissolves in 5N hydrochloric acid. Acetylation gave acetyl-(+)-lunacridine, colourless needles from light petroleum (b.p. 40–60 °C) which melted at 88–89 °C (mixed m.p. with (+)-lunacridine *c*. 75 °C) (Found: C, 65·7; H, 7·3; CH_3CO, 12·9%; $[\alpha]_D^{20}$ +66·7° (*c*, 0·91 in ethanol). Calc. for $C_{19}H_{25}O_5N$: C, 65·7; H, 7·2; CH_3CO, 12·4%). Ultraviolet absorption of (+)-lunacridine in ethanol solution: $\lambda_{max.}$ 238·5, 258, 284, 292, and 330 mμ (log ε 4·38, 4·40, 3·92, 3·89, and 3·52 respectively), $\lambda_{min.}$ 245, 274, 289, and 310 mμ (log ε 4·32, 3·86, 3·89, and 3·35 respectively).

(*d*) (+)-*Lunacridine from* (—)-*Lunacrine*

(i) A solution of (—)-lunacrine (0·5 g) and dimethyl sulphate (5 g) in chloroform (40 ml) was refluxed for 2 hr as described by Dieterle and Beyl (1937). After removal of the solvent the residue was heated with excess aqueous potassium hydroxide (30%), for 15 min, cooled, and extracted with chloroform. Crystallization of the residue after evaporation of the chloroform, first from light petroleum, then from aqueous methanol, gave (+)-lunacridine, colourless needles, m.p. 84·5–85 °C, alone or mixed with the specimen obtained from (—)-lunasine (Found: C, 67·1; H, 7·5%; $[\alpha]_D^{20}$ +30·2° (*c*, 1·01 in ethanol). Calc. for $C_{17}H_{23}O_4N$: C, 66·9; H, 7·5%).

(ii) To a solution of (—)-lunacrine methiodide (50 mg) in water (8 ml) was added, at room temperature, aqueous sodium hydroxide (20% ; 0·5 ml). The solution became cloudy at once and on standing deposited crystals of (+)-lunacridine (30 mg), m.p. 83–84·5 °C, without recrystallization ; the mixed m.p. with (+)-lunacridine from (—)-lunasine was likewise 83–84·5 °C.

(e) *Alkaline Hydrolysis of (—)-Lunacrine*

A solution of (—)-lunacrine (0·8 g) and potassium hydroxide (5 g) in ethanol (50 ml) was refluxed for 8 hr and the ethanol then replaced by water. After extraction with chloroform which yielded only a trace of material, the aqueous alkaline solution was saturated with carbon dioxide and the precipitated solid extracted with chloroform. The residue after removal of the solvent crystallized from aqueous ethanol as colourless needles (0·6 g), m.p. 156–157 °C (Found : C, 65·8; H, 7·3; N, 4·8; CH_3O, 10·3; $CH_3(N)$, 5·3% ; $[\alpha]_D^{21\cdot5}$ —83° (*c*, 1·90 in ethanol). Calc. for $C_{16}H_{21}O_4N$: C, 66·0 ; H, 7·3 ; N, 4·8 ; CH_3O, 10·7 ; $CH_3(N)$, 5·2%). The phenol (II) is only sparingly soluble in 5N hydrochloric acid.

(f) *Action of Hydrochloric Acid on (+)-Lunacridine*

A solution of (+)-lunacridine (1·0 g) in 5N hydrochloric acid (100 ml) was refluxed for 6 hr, diluted, basified, and extracted with chloroform. After removal of the chloroform, the residue (0·92 g) was dissolved in a small volume of methanol, the solution poured into 2N hydrochloric acid, and the methanol removed under reduced pressure. The acid-insoluble material was again treated in this way, the acid solutions combined, washed with benzene, and basified. Chloroform extracted 0·32 g base which, after crystallization from light petroleum and from aqueous methanol, gave (+)-lunacrine, m.p. 116–117·5 °C, mixed m.p. with (—)-lunacrine, 145–146 °C, $[\alpha]_D^{20\cdot5}$ +49·8° (*c*, 1·44 in ethanol) (Found : C, 70·6 ; H, 7·0 ; N, 5·1%. Calc. for $C_{16}H_{19}O_3N$: C, 70·3 ; H, 7·0 ; N, 5·1%). The infra-red spectrum was identical with that of (—)-lunacrine (Fig. 2). (+)-Lunacrine picrate, yellow needles from methanol, melted at 210–211 °C. The mixed m.p. with (—)-lunacrine picrate was 212–213·5 °C (Found : C, 52·4 ; H, 4·4 ; N, 11·1%. Calc. for $C_{16}H_{19}O_3N.C_6H_3O_7N_3$: C, 52·6 ; H, 4·4 ; N, 11·2%).

The weakly basic material (insoluble in 2N hydrochloric acid) was dissolved in light petroleum, and the solution run through a short column of alumina which was then eluted with benzene. The product V crystallized from light petroleum as colourless needles, m.p. 80–81 °C, $[\alpha]_D^{22}$ —56° (*c*, 2·07 in ethanol) (Found : C, 70·2 ; H, 7·0%. Calc. for $C_{16}H_{19}O_3N$: C, 70·3 ; H, 7·0%). V was recovered unchanged (m.p. and mixed m.p.) after refluxing with 10% ethanolic potassium hydroxide for 20 hr.

(g) *Action of Hydrochloric Acid on the Phenol (II)*

The phenol (II ; 2·5 g) was refluxed with 5N hydrochloric acid (100 ml) for 19 hr and the reaction mixture worked up as in (*f*). The acid-soluble fraction (0·4 g), crystallized from aqueous methanol and from *cyclo*hexane, gave a mixture of (+)- and (±)-lunacrine, m.p. 117–135 °C, $[\alpha]_D^{21\cdot5}$ +39·5° (*c*, 2·46 in ethanol). A mixture of 5 parts of this material with 4 parts (—)-lunacrine melted at 141–143·5 °C. The infra-red spectrum (Nujol mull) of the material m.p. 117–135 °C was essentially the same as that of pure (+)- or (—)-lunacrine, but differed in some minor features. When measured in CS_2 solution the spectra were identical.

The weakly basic fraction (2·2 g) was dissolved in light petroleum and chromatographed on alumina. Elution with benzene and crystallization from light petroleum gave colourless needles, m.p. and mixed m.p. with V prepared from lunacridine 79·5–81 °C, $[\alpha]_D^{22}$ —55·5° (*c*, 2·36 in ethanol).

(h) *4-Methoxy-1-methylquinolinium Iodide*

(i) 4-Methoxyquinoline (0·9 g) was dissolved in methyl iodide (8 ml) and allowed to stand at room temperature. The methiodide, which separated rapidly, crystallized from methanol–ether as pale yellow needles, m.p. 133–134 °C (decomp.). Clemo and Perkin (1924) describe the methiodide as pale yellow plates (from methanol) which become opaque on drying on the water-bath and melt with decomposition at 145 °C.

(ii) A solution of 4-methoxy-1-methylquinolinium iodide (0·77 g; prepared, as described above, from 4-methoxyquinoline) in water (12 ml) was made alkaline by the addition of aqueous sodium hydroxide (20%; 5 ml) when an oily precipitate formed immediately. The mixture was extracted with chloroform and the chloroform evaporated. Crystallization of the residue (0·45 g) from water gave 1-methyl-4-quinolone, colourless needles, m.p. after drying 151–152 °C, alone or mixed with an authentic specimen. The picrate, yellow needles from methanol, melted at 231–232 °C alone or mixed with authentic 1-methyl-4-quinolone picrate.

(iii) 1-Methyl-4-quinolone (0·32 g) was dissolved in methyl iodide (3 ml) with warming and allowed to stand. The methiodide, which began to separate after 3 hr, crystallized from methanol–ether as pale yellow needles, m.p. 135–136 °C (decomp.) (Found: C, 43·6; H, 4·1%. Calc. for $C_{11}H_{12}ONI$: C, 43·9; H, 4·0%). A mixture with the specimen prepared from 4-methoxyquinoline melted at 134–135 °C (decomp.). The infra-red spectra of the two specimens were identical.

(*i*) *Methylacronycidinium Iodide*

A solution of acronycidine (0·5 g) in methyl iodide (5 ml), after boiling for 2 min and standing, slowly deposited two kinds of crystal—colourless prisms and yellow needles. The two were separated manually and the yellow needles twice recrystallized from ethanol–ether. Methylacronycidinium iodide was obtained as a yellow microcrystalline powder which did not melt until 171–173 °C though the appearance changed at *c.* 130 °C. It is soluble in water (Found: C, 44·8; H, 4·3%. Calc. for $C_{16}H_{18}O_5NI$: C, 44·5; H, 4·2%).

The colourless prisms which separated with the methiodide were crystallized from benzene–*cyclo*hexane and identified (m.p. and mixed m.p.) as *iso*acronycidine. Methylacronycidinium iodide was rapidly converted to *iso*acronycidine, identified by m.p. and mixed m.p., either by basification of an aqueous solution or by heating to 170 °C. When acronycidine was heated with methyl iodide in a sealed tube at 100 °C the reaction product was essentially *iso*acronycidine. No methiodide could be detected, but a small amount of nor*iso*acronycidine (m.p. and mixed m.p.) was isolated.

Attempts to prepare the methiodide from *iso*acronycidine led only to recovery of the starting material.

III. Acknowledgments

The author is indebted to Dr. S. M. Goodwin for information concerning the progress of the work on *Lunasia amara*, to Dr. L. J. Webb for the provision of plant material, and to Dr. J. B. Willis and Mr. A. Triffett for infra-red spectra. The technical assistance of Mr. R. Johnstone and Mr. L. W. Smith is acknowledged with gratitude.

IV. References

Adams, (Mrs.) W. J., and Hey, D. H. (1951).—*J. Chem. Soc.* **1951**: 1521.

Boorsma, W. G. (1904).—*Bull. Inst. Bot. Buitenz.* **21**: 8.

Brown, R. F. C., Hobbs, J. J., Hughes, G. K., and Ritchie, E. (1954).—*Aust. J. Chem.* **7**: 348.

Clemo, G. R., and Perkin, W. H. Jr. (1924).—*J. Chem. Soc.* **125**: 1608.

Deulofeu, V., and Bassi, D. (1952).—*An. Asoc. Quim. Argent.* **40**: 249.

Dieterle, H., and Beyl, H. (1937).—*Arch. Pharm. Berl.* **275**: 174.

Goodwin, S., and Horning, E. C. (1959).—*J. Amer. Chem. Soc.* **81**: 1908.

Goodwin, S., Shoolery, J. N., and Johnson, L. F. (1959).—*J. Amer. Chem. Soc.* (in press).

Goodwin, S., Smith, A. F., and Horning, E. C. (1957).—*J. Amer. Chem. Soc.* **79**: 2239.

Grundon, M. F., and McCorkindale, N. J. (1957).—*J. Chem. Soc.* **1957**: 2177.

Johnstone, R., Price, J. R., and Todd, A. R. (1958).—*Aust. J. Chem.* **11**: 562.

Lahey, F. N., Lamberton, J. A., and Price, J. R. (1950).—*Aust. J. Sci. Res.* A **3**: 155.

Lewin, L. (1897).—"Lehrbuch der Toxikologie." 2nd Ed. p. 271. (Urban & Schwarzenberg: Vienna and Leipzig.)

Openshaw, H. T. (1953).—" Quinoline Alkaloids, other than those of Cinchona." In " The Alkaloids." (Eds. R. H. F. Manske and H. L. Holmes.) Vol. 3. p. 66. (Academic Press Inc.: New York.)

Price, J. R. (1956).—" Alkaloids Related to Anthranilic Acid." In Zechmeister's " Progress in the Chemistry of Organic Natural Products." Vol. 13. p. 302. (J. Springer: Vienna.)

Price, J. R. (1958).—" A Novel Type of Naturally Occurring Quaternary Base." In " Current Trends in Heterocyclic Chemistry." (Eds. A. Albert, G. M. Badger, and C. W. Shoppee.) p. 92. (Butterworths Scientific Publications: London.)

Price, J. R., and Willis, J. B. (1959).—*Aust. J. Chem.* **12** (in press).

Steldt, F. A., and Chen, K. K. (1943).—*J. Amer. Pharm. Ass.* (*Sci. Ed.*) **32**: 107.

Wirth, E. H. (1931).—*Pharm. Weekbl.* **68**: 104.

STUDIES ON ARGENTINE PLANTS*

XVIII. MULTIFLORINE, THE ALKALOID FROM LUPINUS MULTIFLORUS LAM.

By J. Comin† and V. Deulofeu†

[*Manuscript received April 24, 1959*]

Summary

From the seeds of *Lupinus multiflorus* Lam., a plant growing in north-eastern Argentina, an alkaloid $C_{15}H_{22}ON_2$ has been isolated and named multiflorine. It is identical with the base LV-1 isolated in Australia from the seeds of *Lupinus digitatus* Forsk. by Crow and Riggs (1955). Most of its reactions and properties are explained by accepting a 2-dehydro-4-oxosparteine structure for the alkaloid.

I. Introduction

Lupinus multiflorus Lam. is a plant that grows wild in the north-eastern part of Argentina, along the Parana and Uruguay rivers. Extracts from its seeds gave a strong alkaloidal action, and a base $C_{15}H_{22}ON_2$ was isolated, melting at 107–108 °C. It had $[\alpha]_D^{22}$ −317° (methanol), the highest rotation observed for a lupin alkaloid, and was named multiflorine. The properties of multiflorine were very similar to those of base LV-1, isolated by Crow and Riggs (1955) from the seeds of *L. varius* L. in Australia, and about the structure of which Crow (1958) had already published some information. The identity of the two bases has now been confirmed by mixed melting point and infra-red spectrum.

In Crow's (1959) paper, work on the alkaloid from *L. digitatus* Forsk. is reported, and through his kind cooperation it is possible to report simultaneously our own work with multiflorine which confirms his structure for base LV-1.

II. Reactions and Structure of Multiflorine

Multiflorine is a monoacid base with a maximum at 326 mμ in its ultra-violet spectrum, showing the presence of more than one chromophoric group and conjugation between them. The infra-red spectrum has a very strong carbonyl band at 1630 cm^{-1} and a similar intense band at 1580 cm^{-1}, which must derive from a carbon to carbon double bond (Marion, Ramsay, and Jones 1951).

Catalytic hydrogenation of multiflorine in ethanol and in 1N hydrochloric acid gave an absorption of 2–3 mols of hydrogen per mol of base. Paper chromatography revealed that the hydrogenation product was heterogeneous, and that not less than two compounds were always formed. From the hydrogenation products in 1N hydrochloric acid, (−)-sparteine was isolated showing

* For Part XVII of this series see *Tetrahedron* (1959, in press).

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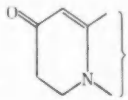
the fundamental structure of multiflorine. Hydrogenation in ethanol gave a compound showing a strong hydroxyl band in the infra-red, while the bands due to the carbonyl and the double bond of multiflorine have disappeared. This hydroxysparteine does not have the characteristics of a carbinolamine, showing that the carbonyl in multiflorine does not participate in the formation of an amido group, but is rather a normal keto group, which must be conjugated to the double bond and the nitrogen, to explain the maximum at 326 mμ.

The double bond must be located in rings A or D of the sparteine skeleton, and the keto group cannot be placed α- to the nitrogen, as it is not an amide, nor at the β-position, as this would not allow conjugation with the nitrogen nor explain the monobasic character of multiflorine.

The γ-position, with the variants I and II formulated by Crow (1958), remains to locate the keto group and the double bond.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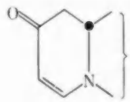
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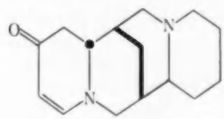

(III)

Structure II is to be preferred, as by controlled oxidation of multiflorine (Galinovsky, Knoth, and Jarisch 1957) there has been isolated the dihydrochloride of a monocarboxylic acid, $C_{13}H_{22}O_2N_2.2HCl.H_2O$, which clearly has two basic centres. Structure I would be expected to yield either a dicarboxylic acid or a monocarboxylic acid with only one basic centre.

A more intense oxidation of multiflorine (Galinovsky, Pöhm, and Riedl 1950) produced succinic acid, glycine, β-alanine, and γ-aminobutyric acid, confirming that the other terminal ring (A or D) of multiflorine does not contain any functional group.

Crow's (1958) isolation, by partial reduction of multiflorine, of an oxosparteine different from the 13-oxosparteine of Galinovsky and Pöhm (1949), locates the keto group and the double bond in ring A of sparteine. From this it is concluded that structure III, assigned by Crow (1959) to base LV-1, is the correct one for multiflorine. In this structure the union of rings A/B is *trans*. This will explain why on dehydrogenation with mercuric acetate, multiflorine gives a faster reaction than with compounds that have a *cis*-union in the quinolizidine rings, although not so fast as sparteine or oxosparteine, which contain a *trans*-quinolizidine ring without any substituent (Fig. 1). This reaction seems to be much influenced by the remaining structure of the molecule and the slower reaction of multiflorine with mercuric acetate is not difficult to understand in this way.

With a *trans*-union of rings A/B it must also be expected that the infra-red spectrum of multiflorine should have the bands at 2700–2800 cm^{-1} which Bohlmann (1958) considers indicative of the existence of a *trans*-quinolizidine structure in the *Lupinus* alkaloids.

Those bands derive from the vibration of C—H bonds and are conditioned to the existence in the nitrogen atom of a solitary pair of electrons in *trans*-

position to the vicinal hydrogens. When the nitrogen is part of a lactam, the bands disappear because of the participation of the solitary electrons in the mesomerism of the group. Multiflorine is the vinylogue of a lactam, and the pair of free electrons must to a certain extent be an interaction with the π-electrons of the double bond and the keto group, which may modify the position of the bands expected for a typical *trans*-quinolizidine ring. In carbon tetrachloride the infra-red spectrum of multiflorine has a band at 2790 cm^{-1} with shoulders

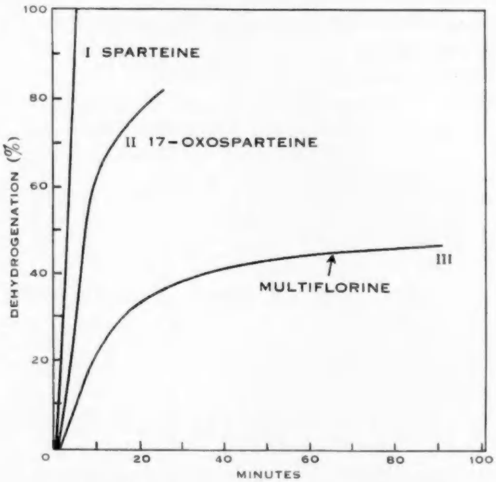


Fig. 1.—Action of mercuric acetate on multiflorine.

at 2740 and 2670 cm^{-1}. Sparteine, with a pure *trans*-quinolizidine ring, had in this region and with the same solvent, a similar spectrum with a band at 2725 cm^{-1} and a shoulder at 2665 cm^{-1}. These data support the existence on carbon 6 of multiflorine of a hydrogen atom with the same relation to the nitrogen as in sparteine and confirm structure II. Compounds with a double bond between carbons 5 and 6, as in structure I, do not present such bands in the spectrum.

III. Experimental

Melting points are uncorrected. Except when noted, descending chromatography was employed on Whatman paper No. 1 with *n*-butanol–acetic acid–water (4 : 1 : 5) as mobile phase.

(*a*) *Multiflorine from* L. multiflorus *Lam.*—Air-dried seeds (5 kg) were well ground, 15 l. methanol added, and stirred for 12 hr, the extraction being repeated once. The combined extracts were evaporated to dryness under reduced pressure, when a pasty residue weighing 770 g was obtained. It was suspended in 2 l. water and hydrochloric acid added carefully to pH 3, when most of the solid matter dissolved.

The suspension was filtered through a bed of " Darco G–60 " and " Filtercel ", and the filtrate extracted four times with 300 ml ethyl ether. It was then made alkaline to pH 12 by the addition of concentrated sodium hydroxide and extracted with 500 ml portions of chloroform until the water phase gave only a faint reaction with Mayer's reagent.

The chloroform extract was treated with a small amount of " Darco G–60 ", filtered, dried, and evaporated in a vacuum, yielding a syrup which crystallized overnight (80 g). The residue was boiled with 800 ml *cyclo*hexane and most of it dissolved, leaving a residue (5 g) which was discarded. On cooling, the solution gave a small amount of an oily precipitate which was also discarded. On scratching, crystals began to appear and the solution was left overnight in a cool room. Multiflorine, m.p. 102–103 °C (25 g), was collected next morning. The filtrate was concentrated to 150 ml and another crop (30 g) of the base, m.p. 104–105 °C, was obtained. Both fractions were pooled and recrystallized twice from *cyclo*hexane, giving 49 g long prisms, m.p. 106–107 °C. For analysis it was recrystallized several times from *cyclo*hexane, m.p. 107–108 °C (Found: C, 73·2, 73·3; H, 9·1, 8·6; N, 11·5, 11·6%. Calc. for $C_{15}H_{22}ON_2$: C, 73·1; H, 9·0; N, 11·4%).

Multiflorine is a monoacid base, with a neutralization equivalent of 250 in water (calc. 246). By titration in acetic acid with perchloric acid the neutralization equivalent found was 122. $[\alpha]_D^{22}$ —317° (*c*, 0·4; methanol); $[\alpha]_D^{24}$ —270° (*c*, 0·4; ethanol); ultraviolet spectrum: $\lambda_{max.}$ 300 mμ; $\log_{10} \varepsilon$ 4·14 (*cyclo*hexane); 326 mμ, $\log_{10} \varepsilon$ 4·16 (ethanol); 320 mμ, $\log_{10} \varepsilon$ 4·14 (0·02N HCl).

On Whatman No. 1 paper, in descending chromatography, with a system *n*-butanol : acetic acid : water (80 : 3 : 17) multiflorine has R_F 0·27; in a similar system with the relations 4 : 1 : 5 the R_F is 0·55. Paper chromatography of the crude extract showed that multiflorine is practically the only alkaloid present in the seeds. It is soluble in water and the usual organic solvents, and can be recrystallized from light petroleum, or preferably from *cyclo*hexane.

In ethanol solution multiflorine gives a red colour with ferric chloride, but the van der Moer reaction, which has been described as characteristic of α-pyridones, is atypical, giving a red-violet colour and not the greenish blue found with cytisine and *N*-methylcytisine. The base is very resistant to acids. After boiling for 5 hr with 2N hydrochloric acid, practically all the multiflorine was recovered.

(*b*) *Multiflorine Picrolonate.*—Prepared by dissolving solid multiflorine in boiling ethanol containing a slight excess of the equivalent amount of picrolonic acid. On cooling, the picrolonate crystallized, and after four recrystallizations from ethanol was obtained as yellow plates, m.p. 204 °C (Found: C, 57·4; H, 6·1; N, 16·1%. Calc. for $C_{15}H_{22}ON_2.C_{10}H_8O_5N_4$: C, 57·0; H, 5·7; N, 16·5%).

No crystalline picrate could be prepared.

(*c*) *Multiflorine Methiodide.*—To 0·50 g multiflorine dissolved in acetone was added 1 ml methyl iodide and the solution boiled for 1 hr. On cooling the methiodide (0·750 g) crystallized out. After several recrystallizations from absolute ethanol it was obtained as long prisms melting with decomposition at 230 °C (slow heating) or 247 °C (fast heating) (Found: C, 47·8, 47·1; H, 6·2, 6·8; N, 7·5, 7·1; I, 33·7%. Calc. for $C_{15}H_{22}ON_2.CH_3I.H_2O$: C, 47·5; H, 6·2; N, 6·9; I, 31·4%).

(*d*) *Hydrogenation in 1N Hydrochloric Acid.—Isolation of* (—)-*Sparteine*. Platinum oxide (0·30 g) was suspended in 20 ml 1N hydrochloric acid and reduced. Multiflorine (1·0 g) dissolved in the same solvent (30 ml) was added and hydrogenated at room temperature and normal pressure for 10 hr.

After filtering the catalyst, the solution was made alkaline with sodium hydrogen carbonate and thoroughly extracted with ethyl ether (25 × 30 ml). The aqueous layer was then alkalinized to pH 12 with concentrated sodium hydroxide solution and extracted five times with 50 ml ethyl ether. The first ether extract, after drying, yielded on evaporation 0·450 g pale yellow oil which gave a spot on paper chromatography coincident with sparteine. It was distilled and the fraction boiling at 130–135 °C (bath temperature) and 0·15 mm pressure collected. The oil obtained had $[\alpha]_D^{27}$ —15·3° (ethanol), and the infra-red spectrum was practically identical with that of (—)-sparteine. The following derivatives were prepared and they gave no melting point depression when mixed with samples prepared from authentic (—)-sparteine: picrate, m.p. 206–208 °C; perchlorate, m.p. 169–170 °C; methiodide, m.p. 240–242 °C (decomp.); 17-oxosparteine, m.p. 84–86 °C.

The second ether extract gave on evaporation, 0·35 g oil, which on paper chromatography gave two spots, one weak with R_F 0·68 (sparteine) and the other with R_F 0·60, which corresponds to the hydroxysparteine described below.

(*e*) *Hydrogenation in Ethanol.—Hydroxysparteine.* Platinum oxide (0·50 g) suspended in 40 ml ethanol was reduced, multiflorine (2·45 g) added, and the base hydrogenated at room temperature and pressure. In 90 min absorption of hydrogen stopped. After filtering the catalyst, the ethanol was evaporated to dryness and the residual oil dissolved in 50 ml 2N hydrochloric acid. The solution was made alkaline to pH 12 with concentrated sodium hydroxide solution. It was extracted with ethyl ether (10×50 ml), and then with chloroform (5×50 ml). After drying, the ether extract, on evaporation, yielded 1·95 g oil, which on paper chromatography gave a spot with R_F 0·60. It was distilled twice and the oil passing at 120 °C (bath temperature) and 0·1 mm collected; $[\alpha]_D^{29}$ −32·5° (*c*, 0·3, ethanol) (Found: C, 72·3; H, 9·7; N, 11·3%. Calc. for $C_{15}H_{26}N_2O$: C, 72·0; H, 10·5; N, 11·2%).

The infra-red spectrum had a strong band at 3333 cm^{-1} (OH), and the bands at 1630 and 1580 cm^{-1} of the unsaturated groups of multiflorine had disappeared.

The chloroform extract, dried and distilled, gave 350 mg oil (R_F 0·38) which was not studied further at that time.

(*f*) *Methiodide.*—The above hydroxysparteine (0·50 g) was dissolved in a mixture of 25 ml acetone and 5 ml methyl iodide and boiled for 3 hr. After standing overnight at room temperature, the crystalline precipitate was filtered and recrystallized from methanol-acetone with the addition of ethyl ether to turbidity, m.p. 258–260 °C (Found: C, 49·6; H, 7·3; N, 7·2; I, 33·3%. Calc. for $C_{15}H_{26}N_2O.CH_3I$: C, 49·0; H, 7·4; N, 7·1; I, 32·4%).

(*g*) *Controlled Oxidation of Multiflorine.*—Multiflorine (1·470 g) was dissolved in 30 ml 2N sulphuric acid, and chromium trioxide (2·5 g) dissolved in 10 ml water added over 2 hr. After leaving 3 hr at room temperature, sulphur dioxide was passed through the solution. When the remaining chromium trioxide was reduced, the solution was heated to boiling and a warm solution of 30 g hydrated barium hydroxide dissolved in 50 ml water, added. The precipitate formed was filtered, washed, and discarded. The barium was eliminated from the filtrate and washings by passing carbon dioxide, and the new precipitate was filtered ("Filtercel") and the filtrate evaporated to dryness. The residue was dissolved in 40 ml 50% ethanol and carbon dioxide passed through the solution. A new precipitate was formed, which was filtered and the filtrate evaporated again to dryness. It was then dissolved in absolute ethanol (1·22 g; 20 ml) and hydrochloric acid added to pH 2. On standing, a crystalline product precipitated, which was filtered and recrystallized from ethanol (95%), long prisms, m.p. 260–264 °C (slow heating), 270–272 °C (fast heating) (Found: C, 47·1; H, 8·0; N, 8·2; Cl, 21·4%. Calc. for $C_{13}H_{22}O_2N_2.2HCl.H_2O$: C, 47·4; H, 7·3; N, 8·6; Cl, 21·7%). Titration revealed the presence of one carboxyl group, besides the two hydrochloric acid molecules. (Neut. equiv.: Found: 111. Calc.: 109).

In the usual system on development with ninhydrin, but on ascending chromatography, it gave a brown spot with R_F 0·10. The product was recovered unchanged after heating for 40 hr with concentrated hydrochloric acid in a closed tube. (Identified by mixed m.p. and paper chromatography.)

(*h*) *Vigorous Oxidation of Multiflorine.*—Multiflorine (1·0 g) was dissolved in 30 ml water, 8 ml concentrated sulphuric acid added, and then with stirring solid chromium trioxide (8 g). The solution was heated in a boiling water-bath for 48 hr and after cooling, the remaining chromium trioxide was reduced with gaseous sulphur dioxide. The green solution was extracted in a continuous extractor with ethyl ether for 48 hr, the ether dried and evaporated. A crystalline residue was obtained (0·180 g) which, recrystallized from acetone, melted at 181–182 °C. No depression was observed on admixture with succinic acid.

The extracted aqueous layer was made alkaline by the addition of barium hydroxide (60 g) dissolved in 200 ml boiling water, when a voluminous precipitate was produced. This was filtered, washed twice with hot water, and the combined filtrate and washings concentrated in vacuum to about 80 ml. The concentrate was extracted continuously with ethyl ether for 48 hr, the extracts

discarded, and the aqueous solution saturated with carbon dioxide. The precipitate formed was filtered and the filtrate evaporated in a vacuum to dryness, the residue taken up in 40 ml 50% ethanol, the solution saturated with carbon dioxide, filtered and evaporated again to dryness. A sample of the solid residue (0·190 g) gave, in ascending chromatography, three ninhydrin positive spots with R_F 0·10, 0·19, and 0·27, which correspond to glycine, β-alanine, and γ-aminobutyric acid.

IV. Acknowledgments

We thank Mr. J. F. Alicino and Dr. N. H. Coy, The Squibb Institute for Medical Research, New Brunswick, N.J., for the microanalyses and infra-red spectra, and Dr. W. D. Crow for the identification of base LV-1 with multiflorine by mixed melting point and simultaneous infra-red spectra.

V. References

Bohlmann, F. (1958).—*Ber. dtsch. chem. Ges.* **91**: 2157.

Crow, W. D. (1958).—"Current Trends in Heterocyclic Chemistry." p. 111. (Butterworths Scientific Publications: London.)

Crow, W. D. (1959).—*Aust. J. Chem.* **12**: 474.

Crow, W. D., and Riggs, N. V. (1955).—*Aust. J. Chem.* **8**: 136.

Galinovsky, F., Knoth, P., and Jarisch, E. (1957).—*Mh. Chem.* **88**: 143.

Galinovsky, F., and Pöhm, M. (1949).—*Mh. Chem.* **80**: 864.

Galinovsky, F., Pöhm, M., and Riedl, K. (1950).—*Mh. Chem.* **81**: 77.

Marion, L., Ramsay, D. A., and Jones, R. N. (1951).—*J. Amer. Chem. Soc.* **73**: 305.

THE ALKALOIDS OF *LUPINUS VARIUS* L.*

III. THE STRUCTURE OF BASE LV-1†

By W. D. Crow‡

[*Manuscript received April* 24, 1959]

Summary

Base LV-1 ($C_{15}H_{22}ON_2$), isolated from the seeds and tops of *Lupinus varius* L., is converted by catalytic hydrogenation to (—)-sparteine. Partial reduction yields a ketonic base $C_{15}H_{24}ON_2$, shown to be 4-oxosparteine, and a study of the physical properties of base LV-1 leads to the alternative structures, Δ^2- and Δ^5-dehydro-4-oxosparteine.

Decision between these alternatives is reached by examination of the nuclear magnetic resonance spectrum, which shows the presence of two vinyl hydrogen atoms, that is, base LV-1 is (—)-Δ^2-dehydro-4-oxosparteine.

Base LV-1 has been shown to be identical with multiflorine, isolated by Comin and Deulofeu (1959) from *L. multiflorus* Lam.

I. Introduction

In the preceding papers of this series (Crow and Riggs 1955; Crow and Michael 1957) the isolation of a new lupin base (base LV-1) was described. In the present paper the determination of the structure of base LV-1 is described.

The alkaloid ($C_{15}H_{22}ON_2$) titrated as a monoacid base (pK_a' 9·25) and formed a monoperchlorate and a monomethiodide. The inference that it was an unsaturated amide of the C_{15}-group (sparteine or matrine) was strengthened by the appearance in the infra-red spectrum of strong bands at 1630 cm^{-1} (amide carbonyl) and 1585 cm^{-1} (conjugated double bond). However, the αβ-unsaturated amides examined by Edwards and Singh (1954) in connection with bakankosine and lycoctonine showed three bands in the 1600–1700 cm^{-1} region, and also differed from base LV-1 in their transparency in the 1500–1600 cm^{-1} region.

The carbon-nitrogen skeleton of base LV-1 was readily established by catalytic hydrogenation over Adams's catalyst in acid solution (Galinovsky and Stern 1944), which gave (−)-sparteine in high yield. The production of sparteine rather than α-*iso*sparteine in this reaction incidentally eliminates the possibility of an 11,12-double bond, and establishes the configuration of base LV-1 at C_{11}.§ Preferential reduction of the carbon-carbon double bond was brought about by partial hydrogenation over palladium charcoal or platinum in glacial acetic acid, but some sparteine was produced, as well as unreacted base LV-1. A more

* Now *L. digitatus* Forsk. (Gladstones 1958).

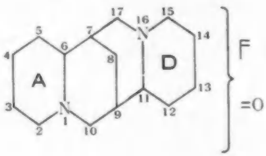
† A preliminary report of some of this work has been published (Crow 1958).

‡ Organic Chemistry Section, C.S.I.R.O. Chemical Research Laboratories, Melbourne.

§ It is of interest to note that no derivative of 14-oxosparteine, in which the configuration at C_{11} is retained, has ever been isolated.

satisfactory method was found in reduction by tin and hydrochloric acid, which afforded in good yield a viscous oily base $C_{15}H_{24}ON_2$, together with only a small amount of sparteine. The reduction base readily formed a crystalline hydrate and monoperchlorate but was not identical with any of the three known sparteine amides (lupanine, aphylline, oxosparteine). Electrometric titration ($pK_a'=11 \cdot 0$) indicated that the reduction base could not be regarded as an amide, which would require $pK_a' \sim 7 \cdot 7$–$9 \cdot 4$. Confirmation that it was in fact a ketone was obtained from the appearance in the infra-red of a carbonyl band at 1720 cm^{-1} (six-membered ring ketone) and preparation of a crystalline oxime, m.p. 169–171 °C. This was clearly not identical with the oxime of 13-oxosparteine prepared by Galinovsky and Pöhm (1949), which had m.p. 244 °C.

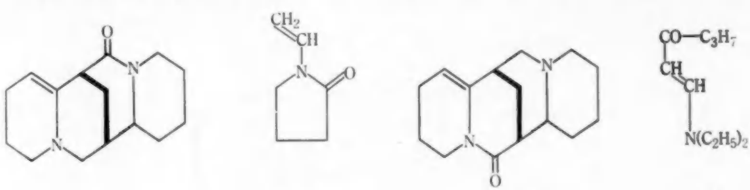
The structure of base LV-1 can thus be expanded as shown in I, with the reservation that the carbonyl group may not occupy positions 2, 10, 13, 15, or 17.



(I)

II. Position of the Carbonyl Group

The fact that base LV-1 was apparently an amide, and yet afforded a ketone on reduction of the carbon-carbon double bond, was almost conclusive evidence for the structural feature —CO—C=C—N. Confirmation of this was obtained by comparison of the ultraviolet spectrum of base LV-1 with those of other possible chromophoric systems (Fig. 1), for example, —CO—NR_2+—C=C—NR_2 (Δ^5-dehydro-17-oxosparteine II), —C=C—NR—CO— (*N*-vinylpyrrolidone III



(II) (III) (IV) (V)

(Breitenbach *et al.* 1956), and aphyllidine (IV), and —CO—C=C—NR_2 (1-diethylaminohex-1-ene-3-one (V); Bowden *et al.* 1946). Only the β-amino-α,β-unsaturated ketone structure V showed similarity to base LV-1. The carbonyl group is therefore γ- to a tertiary nitrogen atom, the only available positions being 4- and 8-. Since stereochemical considerations do not allow either C_7 or C_9 to participate in the α,β-double bond (Bredt's rule), the carbonyl group cannot occupy position 8, and base LV-1 can therefore have either of the structures VI or VII.

At this stage confirmatory evidence can be drawn from the infra-red spectra (Fig. 2) of base LV-1 and the reduction base derived from it (4-oxosparteine). The presence of bands at 2700 and 2800 cm^{-1} in the latter indicate that the C_6–H is axial (i.e. *cis* to the methylene bridge; Bohlmann 1958). The absence of such bands in base LV-1 shows either that N_1 is deactivated, or that a C_5–C_6

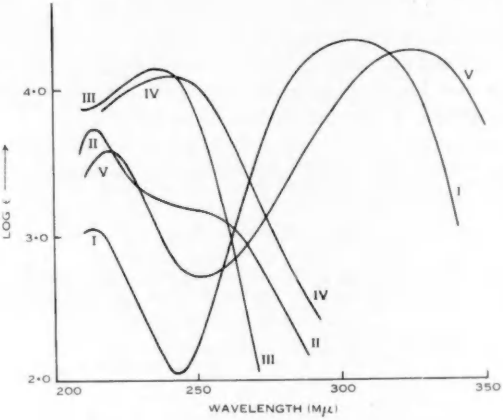
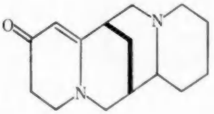
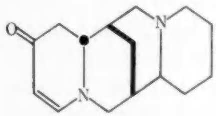


Fig. 1.—Ultraviolet absorption spectra.
I, Base LV-1; II, Δ^5-dehydro-17-oxosparteine; III, *N*-vinylpyrrolidone; IV, aphyllidine; V, 1-aminodiethylhex-1-en-3-one.

double bond is present. In either case it follows of necessity that the carbonyl group occupies the 4-position, thus confirming the previous conclusion without reference to the oxime of 13-oxosparteine. (It was conceivable, although unlikely, that the oxime described in this paper was the other stereoisomer of the oxime of 13-oxosparteine, that is, that the carbonyl group could have occupied position 13 despite the non-identity of the oximes.)



(VI)



(VII)

III. Position of the Double Bond

Having established the structure of base LV-1 as either VI or VII, it remained to decide between the alternatives. The high rotation of the alkaloid (−314°) tends to support the Δ^5-structure VI, also present in Δ^5-dehydrosparteine ($[\alpha]_D^{20}$ −142°), Δ^5-dehydro-17-oxosparteine ($[\alpha]_D^{20}$ −207°), and anagyrine ($[\alpha]_D^{20}$ −165°). However, little real significance could be attached to this, as aphyllidine

(which also has a Δ^5-structure) has $[\alpha]_D^{20}$ $+5°$, and there is no published data available for the alternative Δ^2-structure.

Attempts to split the hydroaromatic ring *A* at the double bond by oxidative methods were generally unsuccessful. Permanganate oxidation resulted only in the production of a neutral compound $C_{15}H_{20}O_2N_2$, presumed to be the 17-oxo

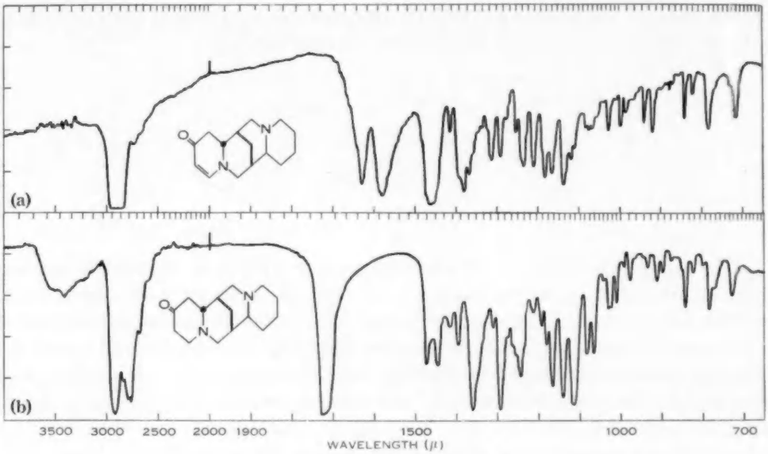
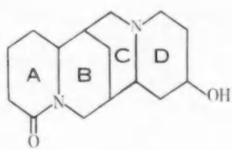


Fig. 2.—Infra-red spectra.
(*a*) Base LV-1 (Nujol mull). (*b*) 4-Oxosparteine hydrate (Nujol mull).

compound by analogy with anagyrine, which is similarly oxidized to anagyramide. No further examination of this reaction product was made, due to shortage of materials. The use of chromic acid under moderate conditions (cf. oxidation of aphyllidine by Galinovsky, Knoth, and Jarisch 1957) led to a mixture of several amino acids from which no pure product could be isolated. Under more drastic



(VIII)

conditions base LV-1 was oxidized to succinic acid, glycine, β-alanine, and γ-aminobutyric acid. Galinovsky, Pöhm, and Riedl (1950) utilized this reaction to determine the position of the hydroxyl group in hydroxylupanine (VIII) and the corresponding hydroxysparteine. The ratio of β-alanine to γ-aminobutyric acid was compared to that obtained in a parallel oxidation of sparteine, and the position of the hydroxyl group in ring D deduced from this. Similar experiments

with base LV-1 showed that the ratio of β-alanine to γ-aminobutyric acid was the same as that obtained with sparteine. Since γ-aminobutyric acid cannot conceivably arise from ring A in base LV-1 (regardless of where the double bond may lie) and the ratio is the same as with sparteine, then it follows that β-alanine is likewise not produced from ring A (i.e. ring D is the sole source of both. In sparteine rings A and D both produce equivalent amounts). The Δ^5-structure VI for base LV-1 would be expected to produce some β-alanine, so these results support the alternative Δ^2-structure VII.

(IX) (X)

The ozonolysis of base LV-1 was next attempted, but no crystalline material could be obtained from the complex mixture of carbonyl compounds which resulted. Theoretically, the two structures VI and VII can be distinguished, as VII would eliminate formic acid after ozonolysis and hydrolysis to yield a secondary amine, whereas VI could only yield a tertiary amide. In practice no secondary amine could be detected, but since the reaction mixture resinified on acid hydrolysis it is probable that the compound IX may have been produced but rapidly underwent autocondensation, for example, to X.

Selective removal of the carbonyl group in base LV-1 was not expected to succeed, and was not in fact achieved. Lithium aluminium hydride afforded (−)-sparteine, as expected, and an attempt to replace the oxygen by sulphur with phosphorus pentasulphide, prior to desulphurization, resulted in the recovery of unchanged starting material.

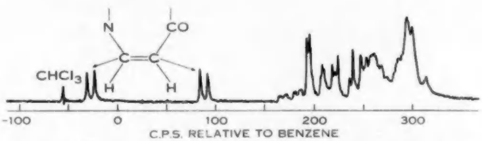
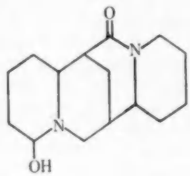


Fig. 3.—Nuclear magnetic resonance spectrum, base LV-1 in $CDCl_3$ at 60 mc.

The possibility of inserting a marker atom at the double bond was next investigated. Addition of a Grignard reagent 1 : 4 to the transoid conjugated system was not to be expected, so attention was directed to bromination. Base LV-1 readily formed a crystalline bromo compound $C_{15}H_{21}ON_2Br$, and there seemed no reason why this should not be smoothly convertible to a bromosparteine by standard procedures. However, attempts to brominate either dehydrosparteine or Δ^5-dehydro-17-oxosparteine yielded complex mixtures from which no suitable reference compounds could be isolated for comparison, so this line of attack was abandoned.

A decision between structures VI and VII was reached by physical methods. The nuclear magnetic resonance spectrum, Figure 3 of base LV-1, showed two distinct doublets corresponding to the two vinyl hydrogens of structure VII. Base LV-1 is thus (—)-Δ^2-dehydro-4-oxosparteine, and offers an interesting comparison to the alkaloid lupanoline XI (Moore and Marion 1953). Lupanoline is formulated as a carbinolamine but undergoes facile dehydration to anhydrolupanoline, which can be hydrated back to the parent base. Base LV-1, on



(XI)

the contrary, is quite stable to hydration, a consequence of the carbonyl–nitrogen interaction which requires the maintenance of the C_2–C_3 double bond. In a preliminary paper (Crow 1958) it was pointed out that structural modification of the type represented by lupanoline and base LV-1 most probably arose by rearrangement of the appropriate *N*-oxide. It is of interest to observe that the preparation of the known Δ^5-dehydrosparteine, as well as the previously unknown Δ^2-isomer, by pyrolysis of *N*-oxides has been reported by Bohlmann (1958).

IV. Experimental

All melting points are corrected. Microanalyses were carried out by the C.S.I.R.O. and Melbourne University Microanalytical Laboratory. Ultraviolet spectra were measured on a Hilger Uvispek spectrophotometer.

(*a*) *Base LV-1*.—This was obtained, as described in the previous paper, as colourless needles, m.p. 108–109 °C, from light petroleum, had $[\alpha]_D^{20}$ —314° (*c*, 0·6 in methanol), and gave a monoperchlorate, m.p. 160–162 °C (decomp.), and a monomethiodide, m.p. 246–248 °C (decomp.). The m.p. of the base was not depressed on admixture with an authentic sample of multiflorine (Comin and Deulofeu 1959), and the infra-red spectra of the two bases were identical.

Alkaline hydrolysis (10% KOH under reflux for 2 hr) failed to give an amino acid, and hydrolysis by aqueous ethanolic HCl (3 hr reflux, 48 hr at 20 °C) returned the unchanged base together with some resinous basic products.

Electrometric titration against 0·1N HCl in water at 20 °C showed the presence of only one basic centre, pK_a' 9·25. Under the conditions of the experiment a second basic centre of pK_a' >3 would have been detected; since it was not possible to prepare a diperchlorate from base LV-1 the presence of a weaker base than this could not be satisfactorily checked in aqueous solution.

(*b*) *Hydrogenation of LV-1*.—(i) Base LV-1 (0·5 g) in 2N HCl (50 c.c.) was added to previously reduced platinic oxide (50 mg) and shaken at room temperature and pressure until absorption of hydrogen had ceased. The uptake was 142 c.c. (corrected to NTP; 3 moles H_2 requires 136 c.c.). The base was recovered by treatment with alkali and ether, and was obtained as an oil (0·45 g) which had R_F 0·51 only (sparteine R_F 0·50), $[\alpha]_D^{20}$ —17·4° (*c*, 2·2 in ethanol) and afforded a perchlorate, m.p. 173–174 °C (from water at pH 7), and a picrate, m.p. 208–209 °C. The m.p.'s

of these derivatives were not depressed by admixture with the corresponding derivatives of authentic (—)-sparteine.

(ii) Base LV-1 (0·5 g) was similarly hydrogenated over platinic oxide in glacial acetic acid (25 c.c.), the reaction being interrupted when the hydrogen uptake was 60 c.c. (1·25 moles H_2—since two competing hydrogenations are occurring, interruption at 1 mole uptake gives a ternary mixture difficult to separate. At 1·25 moles uptake base LV-1 was no longer apparent, and some sparteine was evident). The recovered bases (0·42 g, R_F 0·34, 0·51) were chromatographed over alumina (activity I, 100 g) in peroxide-free ether. Sparteine (R_F 0·51, perchlorate m.p. 172–174 °C) was rapidly eluted, and recovery of the residual bases gave an oily base (0·20 g, R_F 0·34). Solution in light petroleum (b.p. 50–60 °C) resulted in the slow formation of long silky needles of indefinite, m.p. 95–104 °C (Found: C, 67·8; H, 10·7; O, 11·9; N, 10·1%. Calc. for $C_{15}H_{24}ON_2.H_2O$: C, 67·6; H, 9·8; O, 12·0; N, 10·5%). On standing over P_2O_5 the crystals reverted to oil, and recrystallized again on exposure to air. The high percentage of H found is probably due to ready absorption of water, since the formula is confirmed by the preparation of (i) the *monoperchlorate*, obtained from 98% ethanol adjusted to pH 7 by treatment with ammonium acetate, as colourless prisms, m.p. 203–205 °C (Found: C, 51·8; H, 7·4; N, 8·0%. Calc. for $C_{15}H_{24}ON_2.HClO_4$: C, 51·7; H, 7·2; N, 8·0%).

(iii) The oxime was prepared by allowing the base (0·1 g), hydroxylamine hydrochloride (0·1 g), and sodium bicarbonate (0·05 g) to stand for 48 hr in water (5 c.c.). The mixture was then made alkaline and extracted with ether. Concentration of ether and dilution with light petroleum (50–60 °C) afforded the oxime, which, after recrystallization from ether/light petroleum was obtained as colourless prisms, m.p. 161–162 °C (Found: C, 68·7; H, 9·6; O, 6·2; N, 15·6%. Calc. for $C_{15}H_{25}ON_3$: C, 68·5; H, 9·6; O, 6·1; N, 16·0%).

The original base had $[\alpha]_D^{20}$ —33° (*c*, 2·4 in ethanol), and electrometric titration of the monoperchlorate showed pK_a' 11·0 in water at 20 °C.

Reduction of base LV-1 by warming for 1 hr on the water-bath with granulated tin and 5N HCl afforded the same base in near quantitative yield. No sparteine was isolated.

(*c*) *Electrometric Titrations*.—The following compounds were titrated against 0·1N HCl in aqueous solution at 20 °C. Results were checked by titration of a suitable salt (usually the perchlorate) against 0·1N NaOH, and agreed within the limits of experimental error ($\pm$0·1 pK units). The pK_a' values follow.

Sparteine	11·3	17-Oxosparteine	7·7	Δ^5-Dehydro-17-oxosparteine	9·7
4-Oxosparteine	11·0	Tetrahydrothermopsine	9·4		
Lupanine	9·2	Aphyllidine	7·7	Base LV-1	9·3

(*d*) *Δ^5-Dehydro-17-oxosparteine*.—17-Oxosparteine (0·5 g), mercuric acetate (2·0 g), and 5% acetic acid (50 c.c.) were allowed to stand overnight in a stoppered flask. There was only slight precipitation of mercurous acetate, so the mixture was warmed at 50–60 °C for 3 hr, when precipitation was visibly hastened. After cooling and removing the mercurous acetate (1·95 g, corresponding to loss of 0·9 H_2) the mixture was saturated with H_2S, filtered and boiled to remove excess H_2S, and the bases (0·32 g) recovered by treatment with alkali and ether. Δ^5-Dehydro-17-oxosparteine was obtained from ethyl acetate/light petroleum (50–60 °C) or water as colourless needles, m.p. 85–100 °C, and was presumably hydrated. The perchlorate was obtained from methanol as colourless plates, m.p. 213–214 °C (Found: C, 52·0; H, 6·5; O, 23·6; N, 8·1; Cl, 10·0%. Calc. for $C_{15}H_{22}ON_2.HClO_4$: C, 52·0; H, 6·7; O, 23·1; N, 8·1; Cl, 10·2%). The partially hydrated base had $[\alpha]_D^{20}$ —168° (*c*, 0·2 in ethanol). Bohlmann (1958) reports $[\alpha]_D^{20}$ —207° (ethanol) for the distilled base and m.p. 215 °C for the perchlorate.

(*e*) *Oxidation of Base LV-1*.—(i) Base LV-1 (0·5 g) in water (10 c.c.) was cooled ($T \not> 30$ °C) while adding saturated $KMnO_4$ solution. When the colour persisted for 1 min the excess reagent was destroyed by addition of a few crystals of $Na_2S_2O_5$, the precipitate filtered, and washed.

Extraction of the combined filtrates with chloroform/ethanol (4 : 1) afforded a red gum (0·25 g). This was chromatographed over alumina (activity I). Chloroform containing 2% methanol eluted a colourless crystalline solid (0·18 g), which was obtained from acetone/petroleum

(50–60 °C) as colourless needles, m.p. 199–201 °C (Found : C, 69·2 ; H, 7·8 ; O, 12·4 ; N, 10·5%. Calc. for $C_{15}H_{20}O_2N_2$: C, 69·1 ; H, 7·7 ; O, 12·6 ; N, 10·8%) and was presumably Δ^2-dehydro-4,17-dioxosparteine. A strong aqueous solution of the compound was neutral to litmus.

(ii) Base LV-1 (0·20 g) and sparteine (0·20 g) were simultaneously oxidized with chromic acid according to the method of Galinovsky, Pöhm, and Riedl (1950). Succinic acid was isolated from both oxidations, as expected.

Paper chromatography of the amino acids (in two dimensions—butanol/acetic acid and phenol/water) indicated the presence of γ-aminobutyric acid, β-alanine, and glycine. As far as relative abundances of the products were concerned, the two oxidations could not be distinguished.

(iii) Base LV-1 (0·25 g) was oxidized with chromic acid according to the method of Galinovsky, Knoth, and Jarisch (1957). A mixture of several amino acids was produced, and these were separated on Whatman 3 MM paper (butanol/acetic acid) as their methyl esters. However, no crystalline product was obtained.

(*f*) *Miscellaneous Reactions of Base LV-1.*—(i) Base LV-1 perchlorate (0·05 g) was refluxed with powdered KCN (0·02 g) for 2 hr in ethanol (4 c.c.), evaporated to dryness, and the organic material extracted with ether. The oil so obtained was dissolved in cold 0·5% acetic acid (sufficient to give pH ∼6) treated with a few drops of 5% NH_4ClO_4, and the resultant perchlorate filtered off. Recrystallization from methanol by dilution with water gave colourless prisms, m.p. 226–227 °C (decomp.). The compound was unstable, liberating HCN slowly on standing and rapidly on warming with dilute H_2SO_4. Analysis indicated probable incorporation of two nitrile groups (Found : C, 52·3 ; H, 6·4 ; O, 19·9 ; N, 13·3%. Calc. for $C_{17}H_{24}ON_4.HClO_4$: C, 51·0 ; H, 6·3 ; O, 19·9 ; N, 14·0% ; for $C_{16}H_{23}ON_3.HClO_4$: C, 51·5 ; H, 6·5 ; O, 21·4 ; N, 11·2%) presumably by attack at C_4 and C_2.

(ii) Base LV-1 (0·1 g) in chloroform (25 c.c.) was poured into excess bromine in chloroform. The scarlet precipitate was allowed to stand for 10 min, separated off, and shaken with ether and 2% NaOH until it dissolved. Evaporation of the dried ether layer gave colourless needles of the crude bromo compound, which was obtained on recrystallization from acetone as colourless needles, m.p. 169–170 °C (Found : C, 55·9 ; H, 6·8 ; O, 4·9 ; N, 8·3 ; Br, 24·6%. Calc. for $C_{15}H_{21}ON_2Br$: C, 55·4 ; H, 6·5 ; O, 4·9 ; N, 8·6 ; Br, 24·6%). The compound was recovered unchanged after boiling with 10% NaOH for 30 min.

(iii) Base LV-1 (0·05 g) in dry ether (10 c.c.) was allowed to stand overnight with lithium aluminium hydride (0·05 g). After destruction of the excess hydride with a few drops of water the organic layer was evaporated to dryness. The residual oily base gave sparteine perchlorate (m.p. and mixed m.p. with an authentic specimen 172–173 °C).

(iv) Base LV-1 (0·05 g) in ethanol (10 c.c.) was allowed to stand overnight with sodium borohydride (0·10 g). The excess hydride was destroyed by the addition of dilute acid (2N HCl), the mixture basified, and extracted with ether. A small amount (c. 5 mg) of base LV-1 was recovered on evaporation (m.p. 103 °C, undepressed on admixture), but the bulk of the material could not be extracted from the reaction mixture by solvents. Evaporation to small bulk failed to give any tractable product.

(v) Base LV-1 (0·10 g) was heated at 150 °C in the absence of air (N_2 atmosphere) for 15 min with phosphorus pentasulphide (0·50 g). Extraction of the mixture with ether afforded unchanged starting material (m.p. and mixed m.p. with an authentic specimen 101–103 °C) in 70% yield.

V. Acknowledgments

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VI. References

BOHLMANN, F. (1958).—*Ber. dtsch. chem. Ges.* **91**: 2157.

BOWDEN, K., BRAUDE, E. A., JONES, E. R. H., and WEEDON, B. C. L. (1946).—*J. Chem. Soc.* **1946**: 45.

BREITENBACH, J. W., ET AL. (1956).—*Mh. Chem.* **87**: 581.

COMIN, J., and DEULOFEU, V. (1959).—*Aust. J. Chem.* **12**: 468.

CROW, W. D. (1958).—In "Current Trends in Heterocyclic Chemistry." (Butterworths Scientific Publications: London.)

CROW, W. D., and MICHAEL, M. (1957).—*Aust. J. Chem.* **10**: 177.

CROW, W. D., and RIGGS, N. V. (1955).—*Aust. J. Chem.* **8**: 136.

EDWARDS, O. E., and SINGH, T. (1954).—*Canad. J. Chem.* **32**: 683.

GALINOVSKY, F., KNOTH, P., and JARISCH, E. (1957).—*Mh. Chem.* **88**: 143.

GALINOVSKY, F., and PÖHM, M. (1949).—*Mh. Chem.* **80**: 864.

GALINOVSKY, F., PÖHM, M., and RIEDL, K. (1950).—*Mh. Chem.* **81**: 77.

GALINOVSKY, F., and STERN, E. (1944).—*Ber. dtsch. chem. Ges.* **77**: 132.

GLADSTONES, J. S. (1958).—*J. Roy. Soc. W. Aust.* **41** (2): 29.

MOORE, B. P., and MARION, L. (1957).—*Canad. J. Chem.* **31**: 187.

CHEMISTRY OF NON-ENZYMIC BROWNING

IX. STUDIES OF SUGAR MONO-ESTERS OF MALIC ACID FOUND IN BROWNED FREEZE-DRIED APRICOTS

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Summary

Mono-esters of glucose, fructose, and sucrose were prepared by heating the unsubstituted sugar with DL-malic acid. Chromatographic separations showed that four esters were formed from both glucose and fructose and six from sucrose. Three glucose esters were obtained chromatographically pure and two of these gave brucine salts, one crystalline. Periodate oxidation and acid strengths showed that these esters were glucose 1-α-, 6-α-, and 6-β-hydrogen malates. One fructose ester was obtained chromatographically pure and shown to be fructose 6-α-hydrogen malate. Structures were assigned to the other esters on the basis of their $R_{Glucose}$ values and acid strengths. Structures, based on the results of partial hydrolysis with acid, were assigned to three sucrose esters. These results yield presumptive evidence for the identity of the malic acid esters detected in freeze-dried fruit (Anet and Reynolds 1957).

The acids in freeze-dried fruit were determined by elution chromatography using the formate form of an anion exchange resin. The acids and esters in freeze-dried fruit stored at 25, 37, and 50 °C were similarly determined.

I. INTRODUCTION

Mono-esters of glucose, fructose, sorbitol, and sucrose with malic and citric acids have been detected in browned freeze-dried apricots and peaches (Anet and Reynolds 1957). Some of these esters have now been synthesized and their structures deduced from the results of periodate oxidations. The acids and esters in freeze-dried fruit stored at three temperatures have been determined by elution chromatography.

The reaction of malic acid with glucose, fructose, and sucrose yielded two groups of mono-esters derived from the α- and β-carboxyl groups of malic acid (I).† By analogy

$$\begin{array}{l} \mathrm{CHOH.COOH^{\alpha}} \\ | \\ \mathrm{CH_2.COOH^{\beta}} \end{array} \qquad \mathrm{(I)}$$

with α- and β-hydroxybutyric acids, whose pK_a values are 4·12 and 4·29, esterification of the β-carboxyl would be expected (cf. Anet and Reynolds 1957) to produce the stronger group of acids. The formulae below have been assigned accordingly.

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† For nomenclature compare Rodd (1952) where the amide group in asparagine is α or β to the amino group.

The esters were prepared by heating at 100 °C a syrup containing 17 per cent. of water and equal parts of DL-malic acid and the sugar. The α- and β-groups of esters were separated from each other, and from excess malic acid, by displacement chromatography on an anion-exchange resin. Glucose and fructose each gave two α- and two β-hydrogen malates and sucrose gave three esters of each type. The glucose esters were accompanied by condensation products of higher molecular weight.

The glucose α-hydrogen malates were separated from each other, and from the higher condensation products, by means of a cellulose column. One of these esters gave a crystalline, and the other an amorphous, brucine salt. Periodate oxidation of the brucine salts showed that the esters were glucose 1- and 6-α-hydrogen malates. The glucose β-hydrogen malates were also run on cellulose columns. One ester was obtained chromatographically pure and shown, by periodate oxidation, to be glucose 6-β-hydrogen malate. The other ester is presumed from its chromatographic properties to be glucose 1-β-hydrogen malate. The pK_a values of the glucose 6-α- and 6-β-hydrogen malates were 4·2 and 3·9. The 6-malates were slightly weaker acids than the 1-malates. An analogous esterification of glucose, by heating with acetic acid and water, has been described by Duff (1957). He obtained 6-*O*-acetylglucose, together with some di- and triacetylglucose, but apparently no 1-*O*-acetylglucose.

One of the fructose α-hydrogen malates emerged chromatographically pure from the anion exchange columns. Periodate oxidation of the brucine salt showed that this ester was fructose 6-α-hydrogen malate. None of the other fructose esters could be obtained pure. Their chromatographic behaviour suggests that they were fructose 1-α-hydrogen malate and fructose 1- and 6-β-hydrogen malates.

The reaction of sucrose with malic acid resulted in much hydrolysis of sucrose and the sucrose α- and β-hydrogen malates emerged from the anion exchange columns accompanied by the corresponding glucose and fructose esters. Small quantities of the three sucrose β-hydrogen malates were eluted from paper chromatograms. These esters were not hydrolysed by invertase and were thus similar to a sucrose phosphate, esterified in the fructose-6 position, which was not hydrolysed (Leloir and Cardini 1955) by yeast or honey invertase. Partial hydrolysis was effected with 0·1N hydrochloric acid at room temperature, and the products were run on paper chromatograms. This showed that the sucrose moiety was esterified in the fructose-1, fructose-6, or glucose-6 positions.

Three glucose esters, four fructose esters, and two sucrose esters were detected in stored freeze-dried fruit (Anet and Reynolds 1957). The following formulae, based on the order of emergence from ion exchange columns and the R_{Glucose} values, can now be presumed for these compounds: the esters called GM 1–3 by Anet and Reynolds (1957) were glucose 6-α-, 1-α-, and 6-β-hydrogen malates, FM 1–4 were fructose 6-α-, 1-α-, 6-β-, and 1-β-hydrogen malates, and SM 1–2 were the sucrose α- and β-hydrogen malates esterified in the fructose-1 position.

The organic acids and esters in freeze-dried apricots were separated by elution with formic acid on columns of " Dowex 1–X4 ", formate form, following

the method of Palmer (1955). The formic acid in the eluate was blown off with air heated to 50 °C and the residual non-volatile acid was titrated with alkali. Under these conditions formic acid solutions of glucose, fructose, or sucrose yielded respectively 0·45, 0·45, or 0·89 mole of titratable acid per mole of sugar. However, the glucose hydrogen malates were stable during the evaporation and were recovered quantitatively from the column. The behaviour of pure sucrose hydrogen malates was not studied but it would be expected that sucrose esters, if they emerged unchanged from the column, would suffer at least partial

TABLE 1

ACIDS AND SUGAR MONO-ESTERS IN UNBROWNED AND BROWNED FREEZE-DRIED APRICOTS

Sample / Constituents*	Unbrowned	Browned Stored (Weeks) At					
		25 °C		37 °C		50 °C	
		52	108	8	16	2	5
Quinic acid	4·2	—	—	—	—	—	—
Quinic acid plus sugar mono-esters	—	22·6	22·2	20·3	17·3	19·3	20·8
Malic acid	52·0	43·3	38·2	37·0	37·0	43·0	39·0
Citric acid	12·0	—	—	—	—	—	—
Phosphoric acid ..	4·3	—	—	—	—	—	—
Citric plus phosphoric acids	—	10·3	10·2	10·0	10·1	10·9	8·2
Highest probable ester value†	—	19·6	19·2	17·3	14·3	16·3	17·8
Lowest probable ester value‡	—	16·0	15·7	14·1	11·7	13·3	14·5
Decrease in malic plus citric plus phosphoric acids	—	14·7	19·9	21·3	21·2	14·4	21·1

* As mmoles per 100 g dry weight of fruit.

† Obtained by subtracting 3 mmoles (quinic acid) from the values in the second row; this assumes that 30 per cent. of the quinic acid was esterified and therefore lost in the non-ionic fraction.

‡ Obtained by multiplying the highest probable ester value by 2·00/2·45; this assumes equal proportions of sucrose and monosaccharide esters and complete hydrolysis of the sucrose esters in the eluate.

hydrolysis to an equimolar mixture of free and esterified monosaccharide during the formic acid evaporation. Displacement chromatograms (Anet and Reynolds 1957) indicated that freeze-dried fruit which had been stored at 25 °C contained equimolar proportions of sucrose and monosaccharide esters. The true ester value for such a mixture would be 2·00/2·45 times the observed value if the sucrose ester emerged unchanged from the column and if, during the evaporation, it was hydrolysed and the resultant monosaccharide was converted to acids.

The results obtained for unbrowned and browned freeze-dried apricots are shown in Table 1. The separation of citric and phosphoric acids (peak volumes

76 and 80 ml) was not reliable and a total value for the two acids is given for the browned samples. Many more analyses were done on the unbrowned sample permitting the selection of individual values for citric and phosphoric acids. None of the sugar mono-esters could be separated from each other nor from quinic acid. As shown in Table 1, the highest and lowest probable values were calculated for the esters formed in storage. The latter would be further decreased in cases where the esterification of quinic acid was less than the assumed 30 per cent.

In the case of the samples stored for 52 weeks at 25 °C and 2 weeks at 50 °C the agreement between the moles of acid lost and the lower of the calculated ester values suggests that the latter is approximately correct. It has been shown (Anet and Reynolds 1957) that the sucrose in freeze-dried fruit is hydrolysed during storage. Continuing hydrolysis of sucrose and its esters would tend to lower the proportion of sucrose to monosaccharide esters. Consequently the true ester values for the longer storage times probably lie between the upper and lower values shown in Table 1, which would give, for 25 and 50 °C, an increase with time in the ester values.

There was some difference between the trends at different temperatures. After 52 weeks at 25 °C there was, apart from esterification, no loss of acid. After 108 weeks some acid, probably malic, had been converted to some form other than esters. Similarly at 50 °C there was little or no loss after 2 weeks but some loss after 5 weeks. At 37 °C the losses were similar for both 8 and 16 weeks' storage. Livingston (1953) showed that a mixture of malic acid and fructose browned but fructose and hydrochloric acid at the same pH did not. This implies participation of the malic acid in the reaction. The fruit contained a high proportion of fructose so that the loss of malic acid is likely to be due to reaction with fructose or its decomposition products. It is also possible that neutral di- or tri-esters were formed in small quantities and these would not be estimated.

II. Experimental

Microanalyses were carried out by the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory.

(*a*) *Reaction of Malic Acid with Glucose.*—(i) *Separation of Esters by Displacement Chromatography.* D-Glucose (20 g), DL-malic acid (20 g), and water (8 ml) were heated on a steam-bath for 8 hr. The brown syrup produced was dissolved in water (500 ml) and poured onto a set (100, 30, 10, and 3 g) of columns of " Dowex 1-X4 " in the acetate form (cf. Anet and Reynolds 1955). After washing with water to remove excess glucose the esters were displaced with 0·1N hydrochloric acid. The effluent was collected in 10 ml fractions. Each fraction was spotted on Whatman No. 1 paper and run with *n*-butanol–acetic acid–water (4 : 1 : 1 v/v). The papers were treated with silver nitrate–sodium hydroxide (Ash and Reynolds 1954). All fractions contained unidentified compounds with low R_{glucose} values as well as the following esters :

Fraction No.	Ester Present	R_{Glucose} Value of Ester
1–8	Glucose 6-α-hydrogen malate +trace glucose 1-α-hydrogen malate	0·90
9–33	Glucose 1-α-hydrogen malate Glucose 6-α-hydrogen malate	1·13
34–36	Glucose 6-β-hydrogen malate	0·87
37–63	Glucose 1-β-hydrogen malate Glucose 6-β-hydrogen malate	1·00

Tubes 1–33 were combined and evaporated under reduced pressure (bath temperature 40 °C) to a syrup which was dried to constant weight (9·9 g) by repeated addition of acetone. Tubes 34–63, similarly treated, yielded 6·8 g syrup. Total yield 16·7 g, 35·5%.

(ii) *Separation of Glucose 1- and 6-α-Hydrogen Malates on Cellulose Columns.* The syrup (0·5 g) containing glucose 1- and 6-α-hydrogen malates was dissolved in water (2 ml) and applied to a cellulose column (32 by 4 cm). The column was developed with acetone–water (8 : 1 v/v). Other solvents tested and found equally satisfactory were butanol–water (94 : 15 v/v), ethyl acetate–acetic acid–water (8 : 2 : 1 v/v), *n*-butanol–acetic acid–water (4 : 1 : 1 v/v). Glucose 1-α-hydrogen malate (0·03 g) was eluted followed by a mixture of the glucose 1- and 6-α-hydrogen malates (0·16 g) and then by glucose 6-α-hydrogen malate (0·08 g). The compounds with low $R_{glucose}$ values remained on the column.

(iii) *Brucine Salt of Glucose 1-α-Hydrogen Malate.* Glucose 1-α-hydrogen malate (0·021 g) was dissolved in water (5 ml). Brucine (0·026 g) in methanol (5 ml) was added and the mixture warmed to 50 °C and cooled. On evaporating to a small volume crystals of the *brucine salt of glucose 1-α-hydrogen malate* separated. Yield 0·028 g (70%), m.p. 137 °C, $[\alpha]_D^{25}=-5\cdot3°$ (*c*, 0·04, water) (Found: C, 54·5; H, 6·3; N, 3·7%. Calc. for $C_{33}H_{42}O_4N_2.2H_2O$: C, 54·1; H, 6·3; N, 3·6%).

(iv) *Periodate Oxidation of Brucine Salt of Glucose 1-α-Hydrogen Malate.* The salt (50 mg) was dissolved in water (50 ml). Aliquots (5 ml) were pipetted into tubes and 0·2M sodium metaperiodate (1 ml) added. The tubes were shaken, capped, and stood in the dark. Periodate uptake was measured at intervals by adding 0·1N sodium arsenite (5 ml) and back titrating with 0·01N iodine. Formic acid was determined by titration with 0·01N sodium hydroxide (using phenolphthalein as indicator) after addition of ethylene glycol (0·5 ml) to destroy excess periodate. Electrometric titration gave similar results. Formaldehyde was identified by precipitation with dimedone (cf. Reeves 1941).

The ester hydrolysed easily under the conditions of oxidation at room temperature but was stable at +1 °C for approximately 4 hr as shown by the following results:

Time of reaction (hr) ..	2	4	24	48
Sodium periodate consumed (moles)	1·6	1·8	3·6	5·0
Formic acid formed (moles)	1·0	1·1	2·9	5·0

The theoretical requirement for glucose 1-α-hydrogen malate in the pyranose form is 2 moles sodium periodate consumed, with 1·0 mole formic acid produced. The increase in periodate consumption after 4 hr is due to further oxidation occurring after hydrolysis of the ester group.

(v) *Periodate Oxidation of the Brucine Salt of Glucose 6-α-Hydrogen Malate.* Glucose 6-α-hydrogen malate was obtained as a syrup which did not crystallize. The compound ran as one spot on paper in butanol–acetic acid–water (4 : 1 : 1 v/v) and in butanol–water (94 : 15 v/v), $[\alpha]_D^{25}=37\cdot0°$ (*c*, 1·0, water) (Found: C, 40·0; H, 6·2%. Calc. for $C_{10}H_{16}O_{10}$: C, 40·5; H, 5·4%).

The ester (50 mg) was added to brucine (50 mg) in methanol and the mixture was warmed and cooled. Excess solvent was removed under vacuum at 40 °C, the residue extracted with water, and undissolved brucine filtered off. The filtrate was evaporated under vacuum yielding a white non-hygroscopic mass (60 mg), m.p. 115 °C (Found: C, 58·0; H, 6·5; N, 4·3%. Calc. for $C_{33}H_{42}O_4N_2$: C, 57·2; H, 6·1; N, 4·1%).

The periodate oxidation of the brucine salt was carried out at room temperature, constant values being obtained after 2 hr. The moles of periodate consumed and moles of formic acid produced at 2, 4, 8, and 24 hr were respectively 3·9, 4·0, 4·0, 4·2 and 3·8, 4·0, 4·0, 3·9. No precipitate was obtained with dimedone. Theoretical requirements for a glucose 6-ester are 4·0 moles sodium periodate consumed, with 4·0 moles formic acid produced.

(vi) *Periodate Oxidation of Glucose 6-β-Hydrogen Malate.* Glucose 6-β-hydrogen malate was separated from glucose 1-β-hydrogen malate on a cellulose column and it then ran as a single spot on paper. The mixed esters (1·0 g) gave glucose 6-β-hydrogen malate (0·16 g) as a syrup (Found:

C, 40·5; H, 6·0%. Calc. for $C_{10}H_{16}O_{10}$: C, 40·5; H, 5·4%). The periodate oxidation of glucose 6-β-hydrogen malate was carried out at room temperature, constant values being obtained in 3 hr. The moles of periodate consumed and of formic acid produced at 3, 6, 8, and 24 hr were respectively 3·7, 3·9, 3·9, 4·0 and 3·9, 4·0, 3·9, 3·9. No precipitate was obtained with dimedone.

(vii) *pK_a Values of Glucose 6-α- and 6-β-Hydrogen Malates.* pK_a values were determined by electrometric titration of an aqueous solution of the ester with 0·01N sodium hydroxide. The esters were stable up to pH 9–10 but hydrolysed on standing at this pH. The pK_a values for glucose 6-α- and 6-β-hydrogen malates were 4·2 and 3·9.

(*b*) *Reaction of Fructose with Malic Acid.*—(i) *Separation of Fructose Esters on Displacement Chromatograms.* Fructose (20 g), DL-malic acid (20 g), and water (8 g) were heated on a boiling water-bath for 2 hr. The dark brown syrup so formed was dissolved in water (500 ml) and poured onto a set of columns of "Dowex 1" in the acetate form as before. The esters were displaced with 0·1N hydrochloric acid.

Fraction No.	Ester Present	R_{Glucose} Value of Ester
1–6	Fructose 6-α-hydrogen malate	1·24
7–18	Fructose 1-α-hydrogen malate Fructose 6-α-hydrogen malate	1·42
19	Fructose 6-β-hydrogen malate	1·18
20–33	Fructose 1-β-hydrogen malate Fructose 6-β-hydrogen malate	1·32

Fractions 1–6 yielded a syrup (0·8 g) containing fructose 6-α-hydrogen malate. This ester ran as a single spot on paper chromatograms. Fractions 7–18 and 19–33 yielded syrups (2·3 and 2·9 g respectively). Total weight of esters = 6·0 g, 12·8% yield.

(ii) *Periodate Oxidation of the Brucine Salt of Fructose 6-α-Hydrogen Malate.* The brucine salt of fructose 6-α-hydrogen malate was formed by the usual method in 65% yield, as a white non-hygroscopic solid, m.p. 118 °C, $[\alpha]_D^{25}$ —14° (*c*, 2·0, water) (Found: C, 56·6; H, 6·6; N, 4·3%. Calc. for $C_{33}H_{42}O_4N_2$: C, 57·2; H, 6·1; N, 4·1%).

The brucine salt was oxidized with periodate at room temperature, constant values being obtained after 2 hr. The moles of periodate consumed and of formic acid produced at 2, 4, 18, and 24 hr, were respectively 3·0, 3·0, 3·0, 3·1 and 3·0, 2·9, 2·9, 3·0. No precipitate was obtained with dimedone. The theoretical requirements for a fructose 6-ester are 3 moles of oxidant consumed and 3 moles of formic acid produced.

(iii) *pK_a Value for Fructose 6-α-Hydrogen Malate.* The pK_a value of this ester determined by electrometric titration was 3·9.

(*c*) *Reaction of Sucrose with Malic Acid.*—(i) *Separation of Sucrose Esters on Displacement Columns and on Paper.* Sucrose (20 g), DL-malic acid (20 g), and water (8 g) were heated at 100 °C for 2 hr. Considerable colour developed and much sucrose hydrolysed. The syrup was worked up in the usual way. Sprays used on paper chromatograms were silver nitrate–sodium hydroxide and naphthoresorcinol–trichloroacetic acid (Ash and Reynolds 1954).

Fraction No.	Ester Present	R_{Sucrose} Value of Ester
1–8	Sucrose α-hydrogen malates + glucose and fructose α-hydrogen malates	1·53, 0·99, 0·73
9–21	Glucose and fructose α-hydrogen malates	
22–28	Sucrose β-hydrogen malates + glucose and fructose α-hydrogen malates	0·98, 0·73, 0·61
29–43	Glucose and fructose β-hydrogen malates	

Fractions 22–28 were evaporated yielding a syrup (0·8 g) containing sucrose β-hydrogen malates together with glucose and fructose α-hydrogen malates. This syrup was chromatographed on Whatman 3MM paper in butanol–acetic acid–water (4 : 1 : 1 v/v) and the bands containing the sucrose esters were cut out and eluted yielding approx. 10 mg of each of the three sucrose esters.

(ii) *Hydrolysis of Sucrose β-Hydrogen Malates.* Hydrolysis with invertase was unsuccessful. Even after 80 hr treatment no hydrolysis was observed. Standing with 0·1N hydrochloric acid at room temperature for 24 hr caused partial hydrolysis to esters, glucose, and fructose. The $R_{glucose}$ values of the esters so obtained are shown below.

$R_{Sucrose}$ Value of Sucrose Ester	$R_{Glucose}$ Value of Ester Obtained by Partial Hydrolysis
0·98	1·32 (cf. fructose 1-β hydrogen malate)
0·73	1·18 (cf. fructose 6-β-hydrogen malate)
0·61	0·87 (cf. glucose 6-β-hydrogen malate)

(*d*) *Determination of Acids and Esters in Freeze-Dried Apricots by Elution Chromatography.*—(i) *Preparation of Extracts.* Equilibrated freeze-dried apricot purée (browned or unbrowned) (5·00 g) was mixed with water (50 ml) in a water cooled Waring microblendor for 2 min. The mixture was centrifuged at 8000 r.p.m. for 30 min and the clear supernatant decanted and stored at —20 °C. To remove amino acids and amino acid–deoxyfructoses from the samples, acetone dried " Dowex 50 " resin (1·0 g, hydrogen form) and aqueous fruit extract (10 ml) were mixed and allowed to stand overnight when a negative ninhydrin test was obtained. The supernatant was then decanted. Aliquots of 1 or 2 ml were applied to the columns for elution chromatography.

(ii) *Elution Chromatography of Organic Acids.* The organic acids were separated on columns (15 by 0·9 cm) of " Dowex 1-X4 ", 200–400 mesh, formate form, according to the method of Palmer (1955). The formic acid in the effluent could not be blown off completely with cold air, as described by Palmer (1955), but was removed with air heated to 50 °C. Compressed air was passed through a glass coil, 7 in. long, 2½ in. diameter, 8 coils, ¼ in. (i.d.) tubing. This coil was immersed in a water-bath at 50 °C. The elution volumes of the acids present in apricot purée were as follows:

Acid in effluent:	Quinic	Malic	Citric	Phosphoric
Peak volume (ml):	18	44	76	80

(iii) *Elution Chromatography of Sugar Esters.* The sugar mono-esters were eluted in a broad peak at approximately 20 ml and were thus separated from all other acids except quinic acid. No separation of the esters of the different acids was achieved nor could the synthetic glucose 1- and 6-α-hydrogen malates be separated. Quantitative recoveries of the malate esters of glucose were obtained with this method.

(iv) *Identification of Elution Peaks.* The different peaks were identified by paper chromatography after excess formic acid had been evaporated from the samples. The residues were dissolved in a few drops of water and run on Whatman No. 1 paper. Sugar esters were run in butanol–acetic acid–water (4 : 1 : 1 v/v) and developed with silver nitrate–sodium hydroxide. Acids were run in methyl ethyl ketone–cineole–formic acid (53% w/w) (50 : 50 : 36 v/v) and developed by spraying with silver nitrate and heating (Anet and Reynolds 1955).

(v) *Reaction of Sugars with Formic Acid.* Glucose or fructose (1 ml of 2% solution) was mixed with 3·5N formic acid (2 ml) and evaporated at 50 °C in the same way as effluent fractions from the " Dowex 1-X4 ". The residue was titrated with 0·01N sodium hydroxide, 5·0 ml alkali being required. Similarly, sucrose (1 ml of 2% solution) required 5·2 ml 0·01N sodium hydroxide.

(vi) *Samples Used.* Freeze-dried apricot purée (moisture content 3·8%), prepared as previously described (Anet and Reynolds 1957), was stored at —20 °C. Samples were equilibrated to 70% R.H. over saturated aqueous strontium chloride and then stored at 50 °C for 2 and 5 weeks, at 37 °C for 8 and 16 weeks, and at 25 °C for 52 and 108 weeks. Before extraction all samples were re-equilibrated to constant moisture content (20%). Results are presented in Table 1. Each value given is the mean of at least three separate determinations.

III. Acknowledgments

The authors are indebted to Mr. B. I. Brown and Mr. A. Hughes for technical assistance.

IV. References

ANET, E. F. L. J., and REYNOLDS, T. M. (1955).—*Aust. J. Chem.* **8**: 267.
ANET, E. F. L. J., and REYNOLDS, T. M. (1957).—*Aust. J. Chem.* **10**: 182.
ASH, A. S. F., and REYNOLDS, T. M. (1954).—*Aust. J. Biol. Sci.* **7**: 435.
DUFF, R. B. (1957).—*J. Chem. Soc.* **1957**: 4730.
LELOIR, L. F., and CARDINI, C. E. (1955).—*J. Biol. Chem.* **214**: 157.
LIVINGSTON, G. E. (1953).—*J. Amer. Chem. Soc.* **75**: 1342.
PALMER, J. K. (1955).—Conn. Agric. Exp. Sta. Bull. No. 589.
REEVES, R. E. (1941).—*J. Amer. Chem. Soc.* **63**: 1476.
RODD, E. H. (1952).—"Chemistry of Carbon Compounds." Vol. 1. Pt. B. p. 1109. (Elsevier Publishing Co.: New York.)

CHEMISTRY OF NON-ENZYMIC BROWNING

X. DIFRUCTOSE-AMINO ACIDS AS INTERMEDIATES IN BROWNING REACTIONS

By E. F. L. J. Anet*

[*Manuscript received March 12, 1959*]

Summary

The visible and ultraviolet spectra of various mixtures stored at 37·5 or 50 °C were compared. Each mixture consisted of an aqueous syrup of sorbitol buffered at pH 5·5, to which an amino acid (0·1M) and one of the following: difructose-glycine, fructose-glycine, or glucose (0·05M) were added.

Difructose-glycine mixtures browned much faster than those containing fructose-glycine, and there was very little browning in the glucose mixtures. The rapid browning with difructose-glycine is attributed to the non-nitrogenous carbonyl decomposition products.

Bisulphite largely eliminated the production of material absorbing light above 240 mμ, except for a chromophore with a maximum at 295 mμ, derived from fructose-glycine.

All the amino acids gave similar browning curves, but the rates varied. The greatest differences occurred in mixtures containing difructose-glycine, γ-aminobutyric acid giving the highest rates.

The results show that diketose-amino acids can be important intermediates in non-enzymic browning reactions.

I. Introduction

The first steps in the "Maillard reaction" or non-enzymic browning in aldose-amine systems are generally accepted as being the formation of glycosylamines which undergo the Amadori rearrangement to give a ketose-amine (*N*-substituted-1-amino-1-deoxyketose). The evidence for this has been reviewed by Hodge (1953) and Hurd and Buess (1956). The nature of subsequent reactions leading to the brown polymer is still obscure. It has recently been shown (Anet 1959) that a ketose-amine containing a secondary nitrogen can react with another molecule of aldose to give a diketose-amine and one such compound (difructose-glycine) was obtained pure and crystalline. Diketose-amines decompose rapidly regenerating the ketose-amine and giving unknown carbonyl compounds. It is now shown that these carbonyl compounds brown rapidly in the presence of amino acids.

Difructose-glycine 1,1′-(carboxymethylimino)bis[1-deoxy-D-fructose] dihydrate was used as the source of the reactive carbonyl compounds. The half-life of difructose-glycine at pH 5·5 is 6 hr at 50 °C and 24 hr at 37·5 °C (Anet 1959). The browning of difructose-glycine with various amino acids was compared with that produced from fructose-glycine, glucose, 5-(hydroxymethyl)-furfuraldehyde, and glyceraldehyde.

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The effect of bisulphite on the browning systems was also studied. Bisulphite inhibits non-enzymic browning (Hodge 1953) but has much less effect on the rate of formation of fructose-glycine (Reynolds 1959) and probably also of difructose-glycine since difructose-glycine has been isolated from mixtures containing bisulphite (Anet 1959).

These reactions were carried out in an aqueous syrup of sorbitol buffered at pH 5·5. Sorbitol was used as an inert diluent to keep the concentration of water as low as possible and yet have all the components in solution.

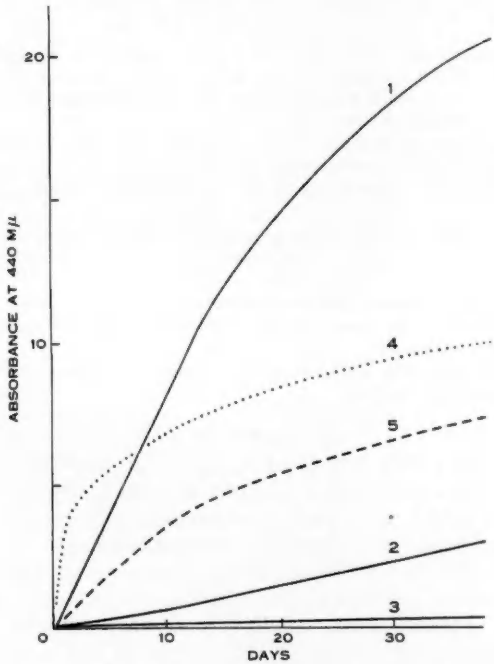

Fig. 1.—Browning at 50 °C of mixtures of glycine (0·1M) and a sugar or derivative (0·05M).
1, Difructose-glycine ; 2, fructose-glycine ; 3, glucose ; 4, glyceraldehyde ; 5, 5-(hydroxymethyl)furfuraldehyde.

II. Experimental

An aqueous sorbitol buffer solution of pH 5·5 was prepared from sorbitol (90% solid ; 200 g), disodium hydrogen phosphate (0·2M ; 28·5 ml), and citric acid (0·1M ; 21·5 ml). Solutions of amino acids (0·1M) were prepared in this buffer. The following amino acids were used : L-α-alanine, γ-aminobutyric acid, L-asparagine, monosodium L-aspartate, and glycine. The γ-aminobutyric acid solution was acidified with citric acid to bring it back to pH 5·5. The glycine mixtures were also studied with the addition of a mole of sodium bisulphite per mole of glycine.

An equal quantity (4 ml) of each amino acid solution was added to stoppered specimen tubes containing 0·2 mmole of difructose-glycine dihydrate, fructose-glycine, or glucose. Four sets of tubes were prepared, two sets were kept at 37·5 °C, and the other two at 50 °C. The fructose-glycine and the glucose dissolved immediately but the difructose-glycine took up to 24 hr to dissolve. Similar mixtures were also made from glycine and glycine bisulphite with glyceraldehyde and 5-(hydroxymethyl)furfuraldehyde.

At intervals samples (0·2 ml) were withdrawn from each tube with a pipette. The pipette, which was calibrated to contain 0·2 ml, was then washed with water (2 ml). The absorptions of the resulting solutions (diluted with water if necessary) were measured at 295 and 440 mμ in a Beckman model DU spectrophotometer. Some full absorption curves down to 220 mμ were also taken on at least one sample from each amino acid. The absorbances shown in the tables and figures were calculated for the original syrup and 1 cm path length.

III. Results and Discussion

The browning (absorbance at 440 mμ) of a mixture of difructose-glycine and glycine was compared with that produced from other substances and glycine (Fig. 1 and Table 1). Difructose-glycine gave more browning than fructose-glycine and much more than glucose. Since the difructose-glycine was completely decomposed very early in these experiments the difference between the browning of difructose-glycine and fructose-glycine represented that due to the non-nitrogenous carbonyl decomposition products from difructose-glycine (Fig. 2). This was much greater than the browning from fructose-glycine. The rates at 37·5 °C were slower than at 50 °C and gave an apparent activation energy of 21,000 cal/mole for the browning of difructose-glycine with glycine.

Table 1

Absorbances at 440 and 295 mμ of mixtures of an amino acid with difructose-glycine, fructose-glycine, or glucose

Wavelength and Storage Time	Sugar	Amino Acid				
		Glycine	α-Alanine	Aspartic Acid	Asparagine	γ-Amino-butyric Acid
440 mμ 9 days at 50 °C	Difructose-glycine	7·6	2·9	3·1	2·4	25·6
	Fructose-glycine ..	0·54	0·32	0·48	0·30	0·90
	Glucose	0·15	0·14	0·17	0·11	0·21
440 mμ 31 days at 37·5 °C	Difructose-glycine ..	6·2	1·8	2·1	1·9	11·6
	Fructose-glycine ..	0·42	0·22	0·23	0·27	0·71
	Glucose	0·13	0·15	0·19	0·20	0·26
295 mμ 9 days at 50 °C	Difructose-glycine ..	50·0	27·0	31·0	32·0	147·0
	Fructose-glycine ..	6·7	6·2	7·5	5·7	9·6
	Glucose	1·0	0·81	1·0	0·84	1·1
295 mμ 31 days at 37·5 °C	Difructose-glycine ..	37·6	16·8	19·7	24·5	65·5
	Fructose-glycine ..	3·6	3·0	3·6	3·9	5·4
	Glucose	1·0	0·75	0·8	1·0	0·95

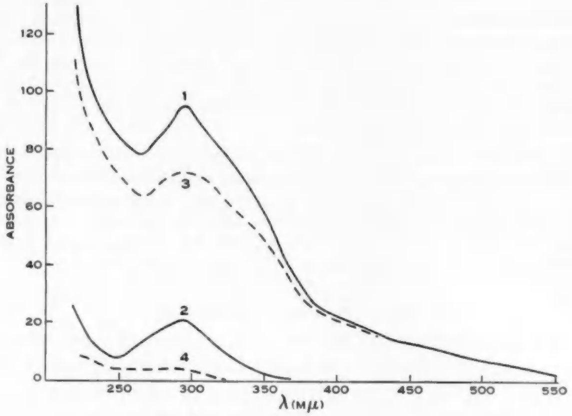

Fig. 2.—Spectra of glycine (0·1M) mixtures after 22 days at 50 °C. 1, Difructose-glycine (0·05M); 2, difructose-glycine (0·05M) and bisulphite (0·05M); 3, carbonyl decomposition products of difructose-glycine. Calculated from 1 minus the absorption due to fructose-glycine (see Fig. 3); 4, carbonyl decomposition products of difructose-glycine in the presence of bisulphite. From 2 less the absorption for fructose-glycine.

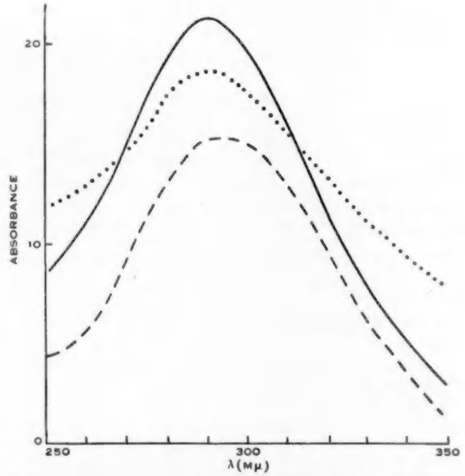

Fig. 3.—Spectra of mixtures after 22 days at 50 °C.
—— Difructose-glycine (0·05M), glycine (0·1M), and bisulphite (0·1M)
- - - - Fructose-glycine (0·05M), glycine (0·1M), and bisulphite (0·1M).
.... Fructose-glycine (0·05M) and glycine (0·1M).

If fructose-glycine decomposed in a similar way to difructose-glycine, the decomposition products from the fructose portion could give rise to the same type of browning. However, spontaneous decomposition of fructose-glycine is very slow compared with that of difructose-glycine (Anet 1959) and is probably not the main pathway of browning from fructose-glycine. Glucose browned very slowly initially and presumably had first to be converted at least to fructose-glycine.

The addition of bisulphite largely eliminated absorption above 240 mμ except for a peak at 295 mμ in the reactions involving difructose-glycine or fructose-glycine (Figs. 2 and 3). The formation of this chromophore was little affected by bisulphite and was probably due to a reaction of fructose-glycine

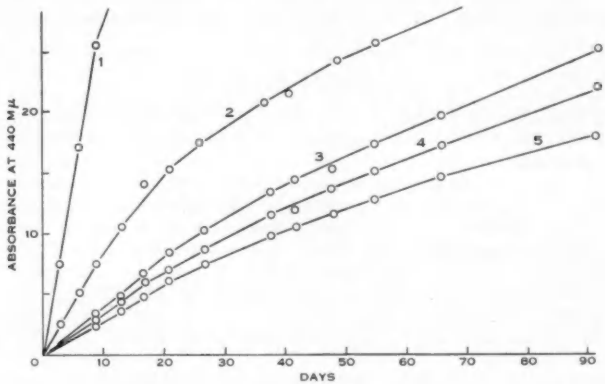

Fig. 4.—Browning at 50 °C of mixtures of difructose-glycine (0·05M) and an amino acid (0·1M).
1, γ-Aminobutyric acid; 2, glycine; 3, aspartic acid; 4, α-alanine; 5, asparagine.

with glycine (Fig. 3). The browning from glyceraldehyde also showed a peak at 295 mμ but it was suppressed by bisulphite. In the case of 5-(hydroxymethyl) furfuraldehyde the 295 mμ region was masked by the strong absorption of the parent compound.

Although 5-(hydroxymethyl)furfuraldehyde is not one of the decomposition products of difructose-glycine at pH 5·5 (Anet 1959) it has been isolated as a minor product from the reaction of glucose and glycine as a syrup (unbuffered, final pH 3·5) at 50 °C (Anet, unpublished data; cf. Wolfrom, Schuetz, and Cavalieri 1949). However, it produced less than half as much brown pigment as the decomposition products of difructose-glycine (Fig. 1). Its part in browning is probably minor except in more acid media where it may be produced in large quantities. Glyceraldehyde gave a very fast initial rate of browning, but there is no evidence that it is produced in Maillard reactions (cf. Hodge 1953).

Mixtures containing other amino acids gave similar results to those for glycine above. Each amino acid gave most browning with difructose-glycine and least with glucose (Table 1). These differences were greatest with γ-amino-

butyric acid and least with asparagine. The browning curves of the various amino acids with difructose-glycine are shown in Figure 4. γ-Aminobutyric acid browned as much as 10 times faster than some α-amino acids at the higher temperature. This effect was smaller when difructose-glycine was replaced by glucose (cf. Gottschalk and Partridge 1950).

In Maillard reactions involving glucose and glycine carried out at pH 5·5, the concentration of difructose-glycine is comparatively low, but its large turnover ensures the formation of a large amount of the carbonyl decomposition products (Anet 1959). Since these decomposition products browned rapidly they, and in turn difructose-glycine, may be the main precursors of the brown pigment. This mechanism should also apply in the case of other aldoses and other primary aliphatic amines but may not be the most important under more acid or alkaline conditions.

IV. Acknowledgments

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V. References

Anet, E. F. L. J. (1959).—*Aust. J. Chem.* **12**: 280.

Gottschalk, A., and Partridge, S. M. (1950).—*Nature* **165**: 684.

Hodge, J. E. (1953).—*J. Agric. Fd. Chem.* **1**: 928.

Hurd, C. D., and Buess, C. M. (1956).—*J. Amer. Chem. Soc.* **78**: 5667.

Reynolds, T. M. (1959).—*Aust. J. Chem.* **12**: 265.

Wolfrom, M. L., Schuetz, R. D., and Cavalieri, L. F. (1949).—*J. Amer. Chem. Soc.* **71**: 3518.

SOLUBLE WOOL PROTEINS

III. OPTICAL ROTATION

By E. F. Woods*

[*Manuscript received January 9, 1959*]

Summary

The optical rotations, $[\alpha]_D$, of three soluble wool keratin derivatives have been measured in a large number of solvents using the sodium D line. A wide range of values was recorded. The values obtained for $[\alpha]_D$ varied from —100° in typical protein denaturing agents such as 8M urea and 6M guanidine hydrochloride to near zero in some organic solvent mixtures. The results are interpreted in terms of the percentage of the polypeptide in the helical configuration. The relation between the optical rotation and the effectiveness of a solvent in disaggregating these proteins is discussed. The optical rotations of the proteins derived from wool appear to be influenced by the methods used to solubilize the proteins.

I. Introduction

Previous papers from this laboratory have considered the behaviour of soluble wool keratin derivatives in several solvent systems (O'Donnell and Woods 1956*a*, 1956*b* ; Harrap and Woods 1958*a*, 1958*b*). In aqueous buffers the protein solutions were very polydisperse and this was attributed to the fact that the proteins exist in various states of aggregation. It appeared possible that the effectiveness of a solvent system in disaggregating the proteins might be related to the configuration assumed by the protein molecules in the solvent. Optical rotation has long been used as a measure of configurational change in proteins and recently attempts have been made to interpret changes in the optical rotation in terms of changes in the helical content of the polypeptide chains (Yang and Doty 1957). In this paper the optical rotations of some soluble keratin derivatives have been measured over a wide range of conditions to determine whether any correlation exists between the ability of a solvent to disaggregate the protein and the optical rotation of the protein in the solvent. During the isolation of the keratin derivatives extensive modification of the protein occurs and an attempt is made to evaluate by means of optical rotation measurements the structural changes which have taken place.

II. Experimental

S-Carboxymethyl kerateine 2 (SCMK 2) was extracted from wool by reduction of the –S–S– bonds with alkaline thioglycollate at 50 °C and reaction of the –SH groups with iodoacetate. α-Keratose was prepared by oxidation of wool with

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peracetic acid, converting the –S–S– groups to $-SO_3$ and extraction of the oxidized wool with ammonia. The preparation of these two proteins is more fully described in a previous paper (Harrap and Woods 1958*a*). *S*-Sulphokerateine 2 (SSK 2) was prepared by conversion of the –S–S– bonds of wool to $-S-SO_3$ by means of cuprammonium hydroxide and sodium sulphite at pH 10 (Swan 1957 ; Woods 1959).

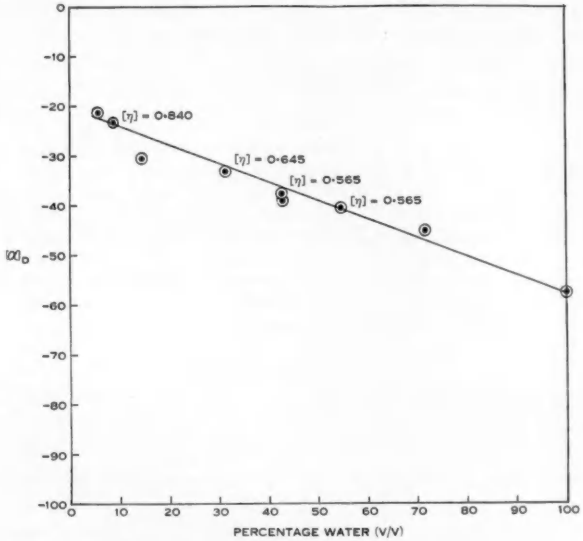

Fig. 1.—Optical rotation and intrinsic viscosity of SCMK 2 in acetic acid–water mixtures.

Optical rotations were measured by means of a Hilger polarimeter with sodium light using a 2 dm tube and protein concentrations between 1 and 2 per cent. The solutions were centrifuged before measurement to remove any insoluble material and the mean of at least 10 and sometimes 20 settings of the polarimeter was taken. Measurements were made at room temperature (20 ± 2 °C). Specific rotations were corrected to equivalent values in water by the equation given by Kauzmann, Walter, and Eyring (1940),

$$[\alpha]=[\alpha]_s \times \frac{n_w^2+2}{n_s^2+2},$$

where $[\alpha]_s$ is the measured specific rotation, and n_w and n_s the refractive indices at 20 °C of water and solvent respectively. This correction has been applied to all the values given in the tables but not to the data of Figures 1 and 2.

Viscosity measurements were made with Ostwald viscometers at 25 ± 0·02 °C. Protein concentrations were determined by means of the semimicro-Kjeldahl

procedure. The percentage of nitrogen was taken at $16\cdot7_5$ for SCMK 2 and 16·5 for α-keratose (Harrap and Woods 1958*a*). The value for SSK 2 was taken to be the same as for SCMK 2. All chemicals used were of A.R. quality or the best commercially available grade.

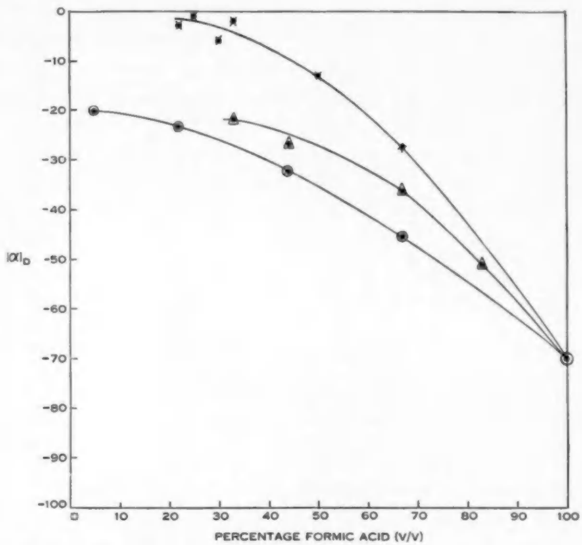

Fig. 2.—Optical rotation of SCMK 2 in formic acid–organic solvent mixtures.

× Formic acid–dimethyl formamide.
△ Formic acid–ethylene dichloride.
⊙ Formic acid–acetic acid.

III. Results

(*a*) S-*Carboxymethylkerateine 2*

(i) *Variations in Preparations of SCMK 2*.—In view of the variations in properties between different preparations of SCMK 2 found in previous work (Harrap and Woods 1958*a*) the specific rotations of 15 preparations were first measured at pH 6·7 in buffer of ionic strength 0·2 (NaH_2PO_4-Na_2HPO_4-NaCl). These gave values for the specific levorotations between 52 and 58°. These preparations were pooled to give 50 g protein which was used for the following work except where otherwise stated. A more restricted series of experiments carried out on a single preparation of SCMK 2 gave almost identical results with those reported here.

(ii) *Aqueous Systems*.—Table 1 gives the values of $[\alpha]_D$ obtained in various aqueous solvents together with the intrinsic viscosity where this has been measured. The state of aggregation of the system is derived from information reported for many of the solvents by O'Donnell and Woods (1956*b*) and Harrap

TABLE 1

OPTICAL ROTATION OF SCMK 2 IN AQUEOUS SOLUTIONS

Solvent Composition	$[\alpha]_D$	$[\eta]$ (dl/g)	State of Aggregation†	Solvent Composition	$[\alpha]_D$	$[\eta]$ (dl/g)	State of Aggregation†
Water (pH 6·2)	—58°			3M Guanidine hydrochloride . .	—81°		Aggregated‡
NaH_2PO_4-Na_2HPO_4-NaCl (I* 0·2 pH 6·7)	—56°	0·38₀	Very aggregated	6M Guanidine hydrochloride . .	—94°	$0{\cdot}45_0$	Disaggregated
$Na_2B_4O_7$-NaCl (I 0·2 pH 9·1) . .	—56°		Aggregated	NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)+0·5 g sodium dodecyl sulphate/g SCMK 2	—62°	$0{\cdot}11_0$	Disaggregated
Na_2CO_3-NaCl (I 0·2 pH 10·8) . .	—65°		Partially disaggregated	5·85M LiBr	—64°	$0{\cdot}17_5$	Aggregated‡
Na_3PO_4-NaCl (I 0·2 pH 11·7) . .	—69°			7·8M LiBr	—54°		
0·1N NaOH	—81°		Disaggregated	Ethanol-water (1 : 2 v/v)	—44°		
4M Urea+NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)	—82°		Aggregated‡	Ethanol-water (1 : 1 v/v)	—38°		
8M Urea+NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)	—102°	$0{\cdot}39_5$	Disaggregated	Acetic acid-water (1 : 1 v/v) . .	—39°		Disaggregated
				Dimethylformamide-water (1 : 1 v/v)	—37°		
10M Urea+NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)	—100°			Dimethylacetamide-water (1 : 1 v/v)	—36°		
				Formamide-water (1 : 1 v/v) . .	—72°		
8M Urea (no added salt pH 7·0)	—100°						
8M Urea (0·1N NaOH)	—104°						

* *I*, ionic strength.

† The information in this column is taken from previously reported sedimentation and light-scattering measurements of O'Donnell and Woods (1956*b*) and Harrap and Woods (1958*a*). Where no comment is given no measurements have been reported.

‡ In these cases the solutions are described as aggregated because they were turbid after centrifuging.

and Woods (1958*a*). Where measurements of molecular size have not been made the protein is described as aggregated if the solution was visibly turbid after centrifuging. Figure 1 relates the specific optical rotation in mixtures of acetic acid and water to the composition of the solvent. Measurements of rotation were made within 30 min of preparation of the solutions and as far as could be ascertained the values listed in Table 1 were attained immediately and no further time effects were evident.

(iii) *Non-Aqueous Systems*.—SCMK 2 was found to be insoluble in most organic solvents and, therefore, in order to study the optical rotation in these solvents the procedure used by Yang and Doty (1957) was followed, that is, the protein was dissolved in formic acid and increasing quantities of the other solvent added. The results are given in Figure 2 and Table 2. Table 2 gives the specific optical rotation in mixtures of equal volumes of formic acid with some organic liquids.

TABLE 2

OPTICAL ROTATION OF SCMK 2 IN ORGANIC SOLVENTS

Solvent Composition		$[\alpha]_D$	Solvent Composition		$[\alpha]_D$
Formic acid		—69°	2-Chloroethanol	+formic acid (1 : 1 v/v)	—23°
Formic acid+0·14M KCl		—68°	Chloroform		—24°
Dichloroacetic acid		—58°	Ethylene dichloride		—27°
Dioxane	+formic acid (1 : 1 v/v)	—16°	*N*-Methylacetamide		—13°
Ethanol		—22°	Dimethylacetamide		—10°
Acetone		—21°	Dimethylformamide		—14°
Acetic acid		—35°			

(iv) *Reversal of Optical Rotation*.—Changes in the optical rotation of aqueous systems appear to be completely reversible. For example, on removal of urea, guanidine hydrochloride, or sodium dodecyl sulphate the rotation reverts to the original value in neutral phosphate buffer. Similarly for the optical rotation in acetic acid–water mixtures shown in Figure 1 the rotation is reversible along the entire curve. However, after solution in strong alkali (0·1N NaOH for 30 min) reversal to pH 6·7 resulted in a decrease in the specific levorotation from 56 to 52°. Standing at pH 11 or 12 for some time also caused a slight decrease in the specific levorotation on reversal to pH 6·7 which suggests that some racemization may occur in alkaline solutions.

(v) *Effect of Chemical Treatment during Preparation of SCMK 2*.—Since SCMK 2 is prepared by chemical modifications of wool some experiments were carried out to determine the effect of these treatments on the specific rotation. The preparative procedure consisted in alkaline reduction at 50 °C and subsequent alkylation of the –SH groups. Optical rotation measurements were done on the –SH protein (kerateine 2) in the presence of thioglycollate at pH 9. $[\alpha]_D$ was $-48°$ and after converting to the *S*-carboxymethyl derivative the value for $[\alpha]_D$ was $-56°$ under the same conditions.

M

In order to study the effect of heat under alkaline conditions SCMK 2 was prepared by alkaline thioglycollate extraction of wool at 2 °C (Harrap, unpublished data). SCMK 2 prepared by this method was electrophoretically similar to that prepared by the usual procedure at 50 °C, but the intrinsic viscosity and slope of the curve relating reduced viscosity and concentration were much higher than the corresponding values for material prepared at 50 °C. In order to study whether racemization occurred on heating this protein in alkali, a solution of SCMK 2 prepared at 2 °C was heated at pH 11 in a water-bath at 50 °C for 2 hr. The optical rotations of the original and the heated material were measured in 8M urea at pH 6·7. It is assumed that all secondary structure of the protein is destroyed in urea. The heating caused the value of $[\alpha]_D$ to change from −108 to −99°. This change in $[\alpha]_D$ is greater than the experimental error and is in the direction to be expected if racemization occurred.

(*b*) *α-Keratose*

(i) *Variability in Preparations and Specific Rotations in Various Solvents*.—The specific rotations of preparations of α-keratose from wool oxidized with peracetic or performic acids were determined in NaH_2PO_4-Na_2HPO_4-NaCl buffer at pH 6·7 and ionic strength 0·2. The specific levorotations of four preparations of α-keratose from peracetic acid oxidized wool were 54, 57, 62, and 68°, and from performic acid oxidized wool 54, 54, 54, 57°. The α-keratose samples

Table 3

Optical Rotation of α-Keratose

Solvent Composition	$[\alpha]_D$	Solvent Composition	$[\alpha]_D$
NaH_2PO_4-Na_2HPO_4-NaCl (*I* 0·2 pH 6·7)	−59°	5·85M LiBr	−63°
0·1N NaOH	−83°	Formic acid	−65°
4M Urea, NaH_2PO_4-Na_2HPO_4-NaCl (*I* 0·2 pH 6·7)	−84°	Formic acid + 0·1M KCl	−64°
8M Urea, NaH_2PO_4-Na_2HPO_4-NaCl (*I* 0·2 pH 6·7)	−97°	Dichloroacetic acid	−54°
3M Guanidine hydrochloride	−87°	Dimethylformamide-formic acid (1 : 2 v/v)	−30°
6M Guanidine hydrochloride	−90°	Acetic acid-formic acid (1 : 1 v/v)	−34°
NaH_2PO_4-Na_2HPO_4-NaCl (*I* 0·2 pH 6·7) + 0·5 g sodium dodecyl sulphate/g α-keratose	−64°		

prepared from performic acid oxidized wool were kindly supplied by Dr. E. O. P. Thompson and Mr. I. J. O'Donnell. Two preparations, prepared from peracetic acid oxidized wool with specific levorotations of 54 and 62°, were pooled and the specific rotations in several solvents determined (Table 3).

(ii) *Effect of Time on Optical Rotation*.—The viscosity of α-keratose solutions has been shown to decrease with time both in aqueous buffer solutions and in formic acid (O'Donnell and Woods 1956*a*; Harrap and Woods 1958*a*, 1958*b*). The change in optical rotation of a neutral aqueous α-keratose solution was less

than 1° in 10 days, during which time there was a 30 per cent. decrease in the reduced viscosity. In formic acid $[\alpha]_D$ changed from $-63 \cdot 2$ to $-60 \cdot 6°$ in 18 days, the reduced viscosity decreasing by more than 50 per cent. in this interval.

(iii) *Effect of Heat at pH 11.*—When heated at pH 11 at 50 °C for 2 hr, the specific rotation when measured in neutral 8M urea was $-92°$ compared with the value of $-97°$ (Table 3) for the unheated material.

(c) S-*Sulphokerateine 2* (*SSK 2*)

Three preparations of SSK 2 gave the following values for $-[\alpha]_D$ in neutral phosphate buffer: 59, 68, 61°. The latter two preparations were pooled and the specific rotations in several solvents determined (Table 4). Rotations were not measured in alkaline solutions because the stability of the $-SSO_3$ group was in doubt (Swan 1957). SSK 2 was also very easily precipitated by organic solvents from a formic acid solution, and was precipitated by high concentrations of lithium bromide.

TABLE 4
OPTICAL ROTATION OF SSK 2

Solvent Composition	$[\alpha]_D$	Solvent Composition	$[\alpha]_D$
NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)	−62°	NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7) + 0·5 g sodium dodecyl sulphate/g SSK 2	−71°
4M Urea, NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)	−77°	Formic acid	−73°
8M Urea, NaH_2PO_4-Na_2HPO_4-NaCl (I 0·2 pH 6·7)	−105°	Dichloroacetic acid	−61°
3M Guanidine hydrochloride	−79°	Acetic acid-formic acid (1 : 1 v/v)	−42°
6M Guanidine hydrochloride	−101°		

IV. DISCUSSION

(a) *Current Views on Interpretation of Optical Rotation of Proteins*

Native globular proteins generally have a specific rotation of −20 to −70° which becomes more negative on denaturation by reagents such as urea or guanidine hydrochloride (Jirgensons 1952*a*, 1952*b*; Simpson and Kauzmann 1953). Linderstrom-Lang and Schellmann (1954) have shown that the rotatory dispersion of native proteins is simple with a value of 250–260 mμ for the dispersion constant, λ_c, which is lowered to near 220 mμ for the denatured state. On the basis of results obtained with polypeptides (Doty, Bradbury, and Holtzer 1956), Yang and Doty (1957) have interpreted the observations on proteins in terms of the fraction of residues in the helical configuration by assuming that the specific rotation is the sum of the intrinsic residue rotation plus contributions due to the helical form. The specific rotation may be taken as inversely proportional to the helical content. Proteins with an average residue weight of 110 in the completely helical form should have a specific rotation, $[\alpha]_D$, in the range 0–23° and the value for the completely denatured form should be 103° more negative (Doty and Lundberg 1957). These generalizations appear qualitatively

correct in spite of the limitations of rotatory dispersion theory (Moffitt, Fitts, and Kirkwood 1957). Some further limitations of these generalizations are also given in the recent papers of Jirgensons (1958*a*, 1958*b*) and Kauzmann (1958).

(*b*) *Discussion of Present Results*

The changes which occur in the specific rotation of the three keratin derivatives in the different solvents are similar and will be discussed together. Although it has not been possible to carry out rotatory dispersion measurements, the results will be interpreted on the basis of Doty's generalizations: namely, an increase in the levorotation (decrease in $[\alpha]_D$) is due to a transition to a more randomly coiled configuration; a decrease in the levorotation (increase in $[\alpha]_D$) is due to a transition to a hydrogen bonded configuration, possibly an α-helix.

Of great interest is the relation between the values obtained for $[\alpha]_D$ and the configuration of the protein as it exists before extraction from the wool fibre. Some of the configurational changes produced in preparing soluble keratin derivatives have been investigated in this paper. It is shown that substitution of the –SH with iodoacetate causes an increase in the levorotation as might be expected from the increase in net charge on substitution. Karush (1957) has shown for reduced serum albumin that the value of $[\alpha]_D$ depends on the nature of the blocking agent and similar considerations would be expected to apply to reduced keratins. The preparation of all three keratin derivatives involves extraction with alkali at pH 10 or higher, and for SCMK 2 heating at 50 °C. The decrease in the levorotation for solutions of SCMK 2 in 8M urea which results from pretreatment at 50 °C suggests that racemization may occur during preparation of the protein. Some amino acids are easily racemized in alkali (Neuberger 1948). In this laboratory Lindley and Swan (unpublished data) have found that when wool is reduced with alkaline thioglycollate and the –SH substituted with iodoacetate, the *S*-carboxymethyl cysteine isolated after hydrolysis is extensively racemized unless the pH and temperature are kept low.

The configurational changes produced by cleavage of the disulphide bonds of wool are difficult to determine. For human serum albumin, Markus and Karush (1957) suggested that elimination of the cystine bridges by reduction allowed the albumin molecule to acquire additional secondary structure whereas reduced bovine γ-globulin appeared less well organized in its secondary structure relative to the native protein. Oxidized human serum albumin has a lower specific levorotation than the native protein but the dispersion constant has the numerical value of a denatured protein (Jirgensons and Ikenaka 1958). For the oxidized A-chain of insulin and oxidized ribonuclease, Schellmann (1958) found a high specific levorotation, low dispersion constant, and a negative temperature coefficient of $[\alpha]_D$, properties which are characteristic of random polypeptide chains and unfolded proteins. Schellmann states that his conclusions cannot be expected to apply to systems in non-aqueous solvents. In non-aqueous solvents Yang and Doty (1957) considered that the development of the helical configuration in oxidized ribonuclease and oxidized bovine serum albumin is aided by the removal of the cystine bridges. Turner, Bottle, and Haurowitz

(1958) conclude that the changes in $[\alpha]_D$ (measured in 88 per cent. formic acid) for a number of proteins following performic acid oxidation could be explained as mainly due to the differences in the contributions to the rotation of L-cysteine and L-cysteic acid, and that conformational changes caused only minor changes in $[\alpha]_D$.* In interpreting the effect on $[\alpha]_D$ of breaking the disulphide bonds of proteins the results obtained will clearly depend on the solvent system in which measurements are made and the possible existence of configurations other than helices or random coils. Hence from specific rotation measurements on the dissolved keratins studied in the present paper it is not possible to determine the configuration of the proteins as they exist in the wool fibre.

The values of $[\alpha]_D$ in neutral solution for the three keratin derivatives are of the same order as those found for many native proteins and indicate that the proteins still have a considerable degree of intramolecular bonding even though the –S–S– bonds have been broken.† Using the calibration given by Yang and Doty (1957) for poly-L-glutamic acid, an average residue weight of 110, and $[\alpha]_D = -100°$ for the completely random form, an estimate of 30–40 per cent. helix is obtained for these proteins in neutral solution. The effect of pH on $[\alpha]_D$ for SCMK 2 and α-keratose can be explained in terms of expansion of the molecules and disruption of the helical configuration due to the increased net charge. Thus there is an increase in the levorotation between pH 9 and 11 corresponding to repression of ionization of ε-amino groups of lysine and ionization of phenolic hydroxyl groups of tyrosine and a further increase above pH 12 corresponding to ionization of the guanidine groups. 8M urea or 6M guanidine hydrochloride causes a large increase in the levorotation for all three keratin derivatives and it is assumed that the molecules are completely random in these solvents. 8M urea and 0·1N sodium hydroxide together are not much different in their effect on $[\alpha]_D$ from 8M urea alone, indicating that the transformation effected by alkali is similar to that effected by 8M urea. Formic acid and 50 per cent. formamide also produce very small increases in the levorotation, whereas dichloroacetic acid has practically no effect on $[\alpha]_D$. The changes observed in urea, guanidine hydrochloride, and alkali are reversible in contrast to the effects observed for many native proteins (Jirgensons 1952*b*).

The results with organic solvents show that in most cases a decrease in the levorotation is observed indicating that the helical configuration is favoured in these solvents. The transition from a randomly coiled form to a helical form is gradual (Figs. 1 and 2), as also reported by Yang and Doty (1957) for other proteins. The keratin derivatives are insoluble in all pure solvents which cause a decrease in the levorotation but soluble in mixtures with formic acid or water. It thus appears that the proteins are less soluble when in the helical configuration,

* Experiments in progress in this laboratory with *S*-carboxymethyl bovine serum albumin indicate that $[\alpha]_D$ for this protein in 98·5 per cent. formic acid is only a few degrees different from the value of $[\alpha]_D$, for the native protein in formic acid (Harrap and Woods, unpublished data). These results do not support the conclusions of Turner, Bottle, and Haurowitz (1958).

† Polarographic estimation of the residual disulphide on these keratin derivatives indicates a disulphide content of approximately 10 μmoles/g compared to 500 μmoles/g for wool (Human and Springell 1959; Leach 1959).

but are more soluble in solvents favouring the randomly coiled form. There appears to be a possible solvent effect on the rotation since the changes produced by adding an organic solvent to the protein dissolved in water are not as great as the changes when the same solvent is added to the protein dissolved in formic acid (compare 1 : 1 v/v mixtures in Tables 1 and 2). Yang and Doty (1957) state that solvent effects for polypeptides in organic solvents may be large.

Concentrated lithium bromide solutions have very little effect on $[\alpha]_D$. These results therefore do not support the conclusions of Harrington and Schellmann (1957) that lithium bromide favours the hydrogen-bonded configuration and is a " contra-denaturant ". Moreover lithium bromide is not a good solvent, forming very turbid solutions with SCMK 2 and α-keratose and precipitating SSK 2 from solution.

There appears to be no simple relationship between the effect of a solvent on $[\alpha]_D$ and its effect on the state of aggregation. Solutions of 8M urea and 50 per cent. acetic acid have opposite effects on $[\alpha]_D$, whereas for the sodium dodecyl sulphate complex $[\alpha]_D$ is only changed by a few degrees (Tables 1, 3, and 4), yet these three solvents disaggregate SCMK 2. Aggregation is most likely due to interaction between side chains. This may be by hydrogen bond formation, van der Waals interaction, hydrophobic bonds, or electrostatic interactions. Proteins exist in a completely random configuration in concentrated urea or guanidine hydrochloride due to rupture of intramolecular hydrogen bonds and it seems likely that intermolecular hydrogen bonds also cannot be formed. The disaggregating properties of sodium dodecyl sulphate can be attributed to the formation of a complex of high charge causing strong electrostatic repulsion between the particles. A similar disaggregating effect is obtained by increasing the charge by raising the pH. In 0·1N sodium hydroxide the levorotation is high, whereas the specific rotation of the highly charged detergent complex is little different to the value for the protein in neutral buffer (Tables 1 and 3). It appears that the long carbon chains of the dodecyl sulphate anions help to stabilize the protein structure and their high charge prevents intermolecular attraction. Solvents such as 50 per cent. acetic acid may produce disaggregation because they favour intramolecularly bonded helical forms, leaving fewer hydrogen bonds available for intermolecular interaction. However, the results with 50 per cent. ethanol do not support this interpretation. Side-chain interactions other than hydrogen bonds are likely to be important in causing aggregation. It appears that in buffers at neutral pH these soluble keratins exist in a configuration that favours intermolecular attraction.

In mixtures of acetic acid and water the intrinsic viscosity increases with increasing proportions of acetic acid in a manner corresponding with the changes in $[\alpha]_D$ (Fig. 1). However, the intrinsic viscosity seems to bear no simple relation to the specific rotation, considering all the results (Table 1). This may be expected since all three proteins exist in various states of aggregation depending on the solvent. Also, if the molecules exist in a partly folded, partly random configuration then marked changes in viscosity without any change in optical rotation may occur due to expansion or contraction of the randomly coiled parts of the molecule. This is exemplified in the case of the addition of salt to formic

acid solutions of SCMK 2 and α-keratose. Practically no change in specific rotation occurs (Tables 2 and 3), yet small amounts of electrolyte were found to cause large charges in intrinsic viscosity (Harrap and Woods 1958*b*). Moreover, if proteins in solution can exist in configurations other than helices or random coils then simple relations between optical rotation and other properties would not be expected.

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VI. References

Doty, P., Bradbury, J. H., and Holtzer, A. M. (1956).—*J. Amer. Chem. Soc.* **78**: 947.
Doty, P., and Lundberg, R. D. (1957).—*Proc. Nat. Acad. Sci. Wash.* **43**: 213.
Harrap, B. S., and Woods, E. F. (1958*a*).—*Aust. J. Chem.* **11**: 581.
Harrap, B. S., and Woods, E. F. (1958*b*).—*Aust. J. Chem.* **11**: 592.
Harrington, W. F., and Schellmann, J. A. (1957).—*C.R. Lab. Carlsberg* **30**: 167.
Human, J. P. E., and Springell, P. H. (1959).—*Aust. J. Chem.* **12**: 508.
Jirgensons, B. (1952*a*).—*Arch. Biochem. Biophys.* **39**: 261.
Jirgensons, B. (1952*b*).—*Arch. Biochem. Biophys.* **41**: 333.
Jirgensons, B. (1958*a*).—*Arch. Biochem. Biophys.* **74**: 57.
Jirgensons, B. (1958*b*).—*Arch. Biochem. Biophys.* **74**: 70.
Jirgensons, B., and Ikenaka, T. (1958).—Abstracts 134th Meeting American Chemical Society. p. 34C.
Karush, F. (1957).—Abstracts 131st Meeting American Chemical Society. p. 53C.
Kauzmann, W. (1958).—*Biochim. Biophys. Acta* **28**: 87.
Kauzmann, W., Walter, J. E., and Eyring, H. (1940).—*Chem. Rev.* **26**: 339.
Leach, S. J. (1959).—*Biochim. Biophys. Acta* **33**: 264.
Linderstrom-Lang, K., and Schellmann, J. A. (1954).—*Biochim. Biophys. Acta* **15**: 156.
Markus, G., and Karush, F. (1957).—*J. Amer. Chem. Soc.* **79**: 134.
Moffitt, W., Fitts, D. D., and Kirkwood, J. G. (1957).—*Proc. Nat. Acad. Sci. Wash.* **43**: 723.
Neuberger, A. (1948).—*Advanc. Protein Chem.* **4**: 298.
O'Donnell, I. J., and Woods, E. F. (1956*a*).—*Proc. Inst. Wool Text. Res. Conf. Aust.* **B**: 71.
O'Donnell, I. J., and Woods, E. F. (1956*b*).—*J. Polym. Sci.* **21**: 397.
Schellmann, J. A. (1958).—*C.R. Lab. Carlsberg* **30**: 439.
Simpson, R. B., and Kauzmann, W. (1953).—*J. Amer. Chem. Soc.* **75**: 5139.
Swan, J. M. (1957).—*Nature* **180**: 643.
Turner, J. E., Bottle, R. E., and Haurowitz, F. (1958).—*J. Amer. Chem. Soc.* **80**: 4117.
Woods, E. F. (1959).—*Aust. J. Biol. Sci.* **12**: 96.
Yang, J. T., and Doty, P. (1957).—*J. Amer. Chem. Soc.* **79**: 761.

THE REACTION BETWEEN THIOGLYCOLLATE AND WOOL PROTEINS

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Summary

Proteins extracted from wool by alkaline thioglycollate containing ^{35}S-thioglycollic acid were radioactive even after exhaustive dialysis.

The amount of thioglycollate binding was dependent to a certain extent on the method of preparation of the proteins. The thioglycollate content of *S*-carboxymethyl kerateine 2 (SCMK 2), a soluble protein fraction prepared from a pH 12·3 extract of wool which had previously been extracted five times at pH 10·5, was about 10 μmoles/g protein. By contrast, another soluble protein fraction designated *S*-carboxymethyl kerateine and prepared from a pH 11·0 extract of wool, contained from 59 to 97 μmoles thioglycollate/g protein, depending on the final stage of preparation. Possible reasons for these variations are discussed.

The strength of binding of the thioglycollate was tested by attempts to displace it with a number of reagents. Several of these were ineffective in removing radioactivity from the wool proteins, whereas disulphide-breaking agents resulted in the removal of all but the equivalent of 3 and 13 μmoles thioglycollate/g SCMK 2 and SCMK respectively.

It is postulated that the bound thioglycollate is present partly in the form of a mixed disulphide composed of half-cystine and half-dithiodiglycollic acid (β-carboxy-β-aminoethyl carboxymethyl disulphide) and partly in an unknown form. The former may arise as an intermediate in the reaction between wool protein and thioglycollic acid. The possible nature of the bound sulphur in the unknown form is discussed.

The thioglycollate content of wool proteins as determined by isotopic means was equal to or less than the thiol + disulphide content measured by amperometric titration with mercurials. It was concluded that SCMK preparations contain up to 19 μmoles cystine and/or cysteine/g protein in addition to any half-cystine residues involved in the mixed disulphide. This conclusion was qualitatively supported by polarographic experiments using the catalytic cobalt wave for the detection of certain sulphur containing compounds.

The importance of the mixed disulphide with regard to heterogeneity and other problems associated with soluble wool proteins is discussed.

I. Introduction

The extraction of wool with alkaline thioglycollate solution results in reduction and solubilization of some of the protein components (Goddard and Michaelis 1934; Gillespie and Lennox 1953). The extent of solubilization has been given detailed attention (Gillespie and Lennox 1955; Lennox 1956), but it was not known whether the reduction or the subsequent coupling reaction with iodoacetate always went to completion. Cystine residues have up to now been regarded as the sole source of the residual disulphide, which has occasionally been found in some preparations (Gillespie 1958). However, it is possible that at

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least some of this disulphide is derived from a mixed disulphide composed of half-cystine and half-dithiodiglycollic acid (β-carboxy-β-aminoethylcarboxymethyl disulphide). Reactions have been described (Kolthoff, Stricks, and Kapoor 1955; Eldjarn and Pihl 1957) in which such mixed disulphides are formed as intermediates in the reduction of disulphides by thioglycollate.

The extraction of wool with ^{35}S-thioglycollic acid, followed by the measurement of the radioactivity of the soluble proteins, provides a method for studying mixed disulphide formation (Springell 1958). The recently developed technique of amperometric titration with neohydrin* (Human 1958) provides a means of investigating the total thiol+disulphide content of the wool protein without prior acid hydrolysis. If amperometrically determined thiol+disulphide exceeds the isotopically determined thioglycollate content, then this would provide evidence for residual cystine and/or cysteine in the protein.

II. Materials and Methods

(a) ^{35}*S-Thioglycollic Acid*

Two samples of the acid with initial specific activities of 7·3 and 6·9 mc/mM were obtained from the Radiochemical Centre, Amersham. The samples, initially containing 7·2 and 6·6 mc respectively, were made up to 100 ml with deoxygenated distilled water, and stored in the frozen state.

(b) *Chemical Preparations*

(i) S-*Carboxymethyl Cysteine*.—This was kindly provided by Dr. J. M. Swan.

(ii) *Dithiodiglycollic Acid*.—The acid was prepared by the oxidation of thioglycollic acid with iodine (Biilman 1905).

(iii) ^{35}*S-Biscarboxymethyl Sulphide*.—A solution of this non-volatile derivative of thioglycollic acid was prepared by coupling a solution of ^{35}S-thioglycollic acid with an excess of iodoacetate, as described below for wool protein extracts.

(iv) *Neohydrin*.—A sample was generously donated by Lakeside Laboratories Inc., Wisconsin, U.S.A.

(c) *Thioglycollate Extraction of Wool*

Solvent-scoured Merino 64's wool top was used in all preparations. The ^{35}S-thioglycollic acid was added in the proportion of 1 ml (40–70 μc) to 29 ml unlabelled 0·1M potassium thioglycollate solution/g wool during the first half-life (87 days). Subsequently the proportion of labelled thioglycollic acid was doubled after each of three half-lives.

S-Carboxymethyl kerateine 2 (SCMK 2) solutions were prepared by the method of Gillespie (1956). Briefly, this consisted of the following six 20 min extractions at 50 °C with 30 ml 0·1M potassium thioglycollate/g wool: five at pH 10·5 and one at pH 12·3. Only the final extract was used; it was brought to pH 8·5, and coupled with a twofold excess of iodoacetic acid at this pH. The pH was adjusted to about 6 after completion of the reaction.

S-Carboxymethyl kerateine (SCMK) solutions were prepared by extracting each g of wool, which was soaked for 1 hr in 0·1M sodium carbonate, with 30 ml

* 3-Chloromercuri-2-methoxypropylurea.

0·1M potassium thioglycollate pH 11·0 for 2 hr at 50 °C as described by Gillespie (1958). Various modifications were however adopted in subsequent steps. The reduced protein was either coupled with iodoacetate, or first acid-precipitated at pH 5·0, redissolved at pH 8·5 in thioglycollate, and then coupled with iodoacetate.

In both types of preparation dialysis was continued for 100 hr or more against running tap water. The final stage of preparation consisted of (i) freeze-drying, (ii) precipitation with 10 per cent. trichloroacetic acid, or (iii) precipitation with acetic acid at pH 4·1. The protein precipitates from (ii) and (iii) were washed and dried with cold ether, acetone, and ether in that order.

In some instances the iodoacetate coupling was omitted altogether, with a view to comparing kerateine (or its ultimate aerial oxidation product) with the *S*-carboxymethyl derivative.

(*d*) *Thioglycollate Treatment of Gelatin*

Commercial gelatin (Davis or Difco) was treated for 2 hr at 50 °C with thioglycollate at pH 11·0 in the same manner as the wool. The solution was coupled with iodoacetate, diluted with an equal volume of water to prevent gelling, and exhaustively dialysed. Portions of the modified gelatin solution were used as such for amperometric titration while the rest was freeze-dried for radioassay.

(*e*) *Treatment of Thioglycollate Extracts*

(i) *Chemical Treatment.*—Cold treatment entailed leaving the protein solutions in contact with the reagents listed in Table 4 for 2 hr at room temperature, followed by dialysis and precipitation with trichloroacetic acid as in (*c*) above. Hot treatments consisted of leaving the protein solution and reagent in a boiling water-bath for 10 min prior to leaving for 2 hr at room temperature.

(ii) *Resin Treatment.*—Protein solutions (10–15 ml) containing up to 30 mg protein were brought to an ionic strength of 0·01 with sodium acetate and passed through a 12×0·9 cm column of "Amberlite IRA–400" or "Dowex 2–X8" in the acetate form as described by Dintzis (1952). The column was washed through with about 30 ml 0·01M sodium acetate, and the total eluate dialysed overnight before freeze-drying.

(*f*) *Measurement of ^{35}S-Thioglycollate Binding*

Radioactivity was measured using a thin end-window Geiger-Müller tube (EHM 2S) coupled to a conventional scaler unit. A standard error of counting of ±5 per cent. was not exceeded. Correction was applied for decay.

Radioactive samples were mounted on aluminium planchettes with a 1 cm^2 area, using sufficient material for counting at infinite thickness.

The amount of ^{35}S in a protein fraction was determined by comparing its radioactivity with that of a protein sample containing known amounts of ^{35}S-biscarboxymethyl sulphide.

As a check on the above technique, known amounts of ^{35}S-thioglycollic acid and labelled protein with an unknown ^{35}S content were converted to barium sulphate and the radioactivities compared. The procedure of Walkenstein

and Knebel (1957) was adopted on a one-tenth scale for this conversion. Both methods gave the same result; thus two preparations with an apparent thioglycollate content of 79 and 68 μmoles/g protein as determined by the routine procedure were shown to contain 78 and 67 μmoles/g protein respectively by the barium sulphate method.

(g) *Polarography and Amperometric Titration*

A Tinsley Model 15 5-recording polarograph was employed; the cell-stand was modified to take the type of cell described by Human and Leach (1956).

For quantitative work the amperometric neohydrin titration procedure (Human 1958) was used. Intact protein material containing from 1 to 5 μmoles disulphide per sample was suitable for estimation. Titrations were carried out in the presence of urea with and without added sulphite. The protein was added in the form of a solution or as a precipitate suspended or dissolved in the urea.

In some instances solutions which had been titrated in the presence of urea and sulphite were exhaustively dialysed against distilled water, and the protein recovered for radioassay by freeze-drying.

The amperometric mercuric chloride titration (Leach 1959) was also used as a check on the neohydrin results, and in some qualitative experiments the catalytic cobalt polarographic wave (Brdička 1933) was employed on HCl-formic acid hydrolysates (Human 1958) of the protein.

Table 1

COMPARISON OF THE EFFECTS OF FREEZE-DRYING AND TRICHLOROACETIC ACID PRECIPITATION ON THE THIOGLYCOLLATE CONTENT OF SCMK 2*

	μmoles Thioglycollate/g Protein	
	TCA† Precipitated	Freeze-Dried
Mean	12·6	10·3
Standard error	3·1	4·0
Number of observations	5	3

* SCMK 2, *S*-carboxymethyl kerateine 2.
† TCA, trichloroacetic acid.

III. Results

(a) *Extent of Thioglycollate Binding*

It became evident that thioglycollate was being retained by soluble wool proteins, although the amount of binding was dependent on the type of preparation. Thus SCMK 2 preparations contained consistently less bound radioactive material than the SCMK preparations. In the case of SCMK 2 the values were similar regardless of whether the protein was obtained by precipitation with trichloroacetic acid or by freeze-drying in the final stage of preparation, and averaged from 10 to 13 μmoles thioglycollate/g protein (Table 1).

On the other hand the SCMK binding values were dependent on details of preparation and varied between 59 and 97 μmoles thioglycollate/g protein (Table 2). In preparations, which had not been precipitated at pH 5·0 prior to iodoacetate coupling, precipitation at the final stage of preparation with trichloroacetic acid resulted in lower thioglycollate binding than either freeze-drying or pH 4·1 precipitation.

Table 2

COMPARISON OF THE EFFECTS OF VARIOUS METHODS OF PREPARATION ON THE THIOGLYCOLLATE CONTENT OF WOOL PROTEINS EXTRACTED AT pH 11·0

Preparations were not precipitated at pH 5·0 before iodoacetate coupling

Type of Preparation	Preparation Method	μmoles TGA*/g Protein		No. of Observations
		Mean	S.E.	
SCMK†	Freeze-dried	86·4	9·2	9
SCMK	pH 4·1 precipitated	97·0	9·8	8
SCMK	TCA‡ precipitated	58·7	10·5	7
Kerateine	TCA precipitated	49·2	13·9	4

* TGA, thioglycollic acid.
† SCMK, *S*-carboxymethyl kerateine.
‡ TCA, trichloroacetic acid.

The effect of pH 5·0 precipitation referred to above is evident from Table 3. The lower thioglycollate content resulting from this treatment is found regardless of the final stage of preparation; this was also confirmed by amperometric titration of the protein solution not subjected to the final precipitation. The difference between the two treatments amounts to 31·0 μmoles/g protein with a standard error of 8·5 μmoles/g protein.

Table 3

EFFECT OF PRECIPITATION AT pH 5·0 ON THE THIOGLYCOLLATE CONTENT OF SCMK*

Treatment	μmoles TGA†/g Protein		No. of Observations
	Mean	S.E.	
Precipitated at pH 5·0..	41·5	6·5	6
Not precipitated ..	72·5	5·6	8

* SCMK, *S*-carboxymethyl kerateine.
† TGA, thioglycollic acid.

The thioglycollate content of trichloroacetic acid-precipitated SCMK is not greatly different from that of kerateine which had been similarly prepared, except for omission of the iodoacetate coupling step (Table 2).

(*b*) *Nature of Thioglycollate Binding*

Preliminary experiments showed that precipitated SCMK could not be freed of thioglycollate by extraction with ethanol, propanol, or pyridine. Subsequently labelled protein solutions were treated with a number of reagents which might be expected to displace the radioactivity and so test further the strength of binding of thioglycollate (Table 4). Radioactivity was largely

TABLE 4

EFFECT OF VARIOUS CHEMICAL TREATMENTS ON THE RETENTION OF RADIOACTIVITY BY WOOL PROTEINS

Preparations were precipitated with trichloroacetic acid

Reagent or Treatment	Final Concentration (%)	μmoles TGA*/g Protein after Treatment	
		SCMK†	SCMK 2†
Untreated sample	—	85·5	5·5
Redialysed H_2O control . .	—	84·5	7·5
Sodium thioglycollate	5	70·0	4·0
Sodium thioglycollate‡	5	18·0	3·5
Sodium carbonate	5	45·5	4·5
Potassium cyanide	§	15·5	3·0
Sodium sulphide	||	16·0	5·0
Sodium sulphite	5	13·0	4·0
Sodium bisulphite	5	17·0	3·5
Lithium bromide	52	85·5	5·0
Urea	24	85·5	7·0
EDTA¶	5	90·0	5·5
Standard error	. . . .	6·2	1·2

* TGA, thioglycollic acid.

Mean of two preparations: SCMK, *S*-carboxymethyl kerateine.

‡ A hot treatment was given for 10 min in this instance; all other treatments were carried out at room temperature for 2 hr.

§ This reagent was prepared by mixing equal volumes of 20 per cent. sodium carbonate with 20 per cent. potassium cyanide. One volume of this solution was added to an equal volume of protein solution.

|| This reagent was prepared by bubbling carbon dioxide through a 10 per cent. solution of sodium sulphide until the pH dropped to 7·5.

¶ EDTA, ethylenediaminetetra-acetic acid disodium salt.

retained following treatment with urea, lithium bromide, ethylenediaminetetra-acetic acid, and cold thioglycollate. In the case of SCMK solutions partial removal was observed following exposure to reagents such as cyanide, sulphide, and hot thioglycollate, while carbonate was less effective. By contrast, SCMK 2 solutions may not have been influenced by these reagents, since the observed reductions are not significant.

It is noteworthy that none of these or other treatments was capable of removing all the attached radioactivity. Thus isotopic assay of a protein solution after reaction with neohydrin and exhaustive dialysis revealed that SCMK 2 retained the equivalent of 6 to 7 μmoles thioglycollate/g protein, and SCMK

TABLE 5

ESTIMATES OF THIOL AND THIOL + DISULPHIDE BY THE AMPEROMETRIC NEOHYDRIN TITRATION COMPARED TO THE THIOGLYCOLLATE CONTENT BY THE ISOTOPIC METHOD

Type and No. of Preparation	Preparation Method	μmoles/g Protein			
		Amperometric		Isotopic	SH + ½SS Other than TGA*
		SH	SH + ½SS	TGA*	
SCMK 2†					
23	Whole solution‖	—	13	9	0
25	Whole solution‖	—	8	11	0
Z82F‡	Whole solution‖	1	11	—	—
Z83F§	Whole solution‖	1	6	—	—
SCMK					
20	Whole solution‖	—	53	54	0
24	Whole solution‖	—	124	97	27
26	Whole solution‖	15	122	84	38
27	Whole solution‖	0	119	83	36
30	Whole solution‖	19	155	132	23
31	Whole solution‖	0	90	79	11
32	Whole solution‖	4	73	68	5
5	pH 4·1 precipitated	—	154	130	24
14	TCA¶ precipitated	—	159	77	82
22	pH 5·0 precipitated whole solution	—	105	103	2

* TGA, thioglycollic acid.
† SCMK 2, *S*-carboxymethyl kerateine 2.
‡ This is a pool of 12 preparations provided by Mr. E. F. Woods.
§ This is a single preparation also provided by Mr. E. F. Woods.
‖ The solutions were freeze-dried for radioassay.
¶ TCA, trichloroacetic acid.

14 to 19 μmoles/g protein. Passage of the SCMK through an anion-exchange resin prior to the neohydrin treatment similarly resulted in the retention of 13 to 16 μmoles/g protein.*

Gelatin was also treated with thioglycollate in the same manner as wool. In spite of various treatments including prolonged dialysis, acid, alkali, and cyanide, as well as resin treatment, the thioglycollate content could not be reduced below 7 to 10 μmoles/g protein.

* It is of interest that Dintzis (1952) failed to remove the last 0·25 equivalent of fatty acid per mole of serum albumin by the anion-exchange resin treatment.

(c) *Neohydrin Titration*

Analysis for thiol and disulphide by amperometric titration using neohydrin was carried out on a number of wool protein preparations, and the results compared with those obtained by the radioisotope technique (Table 5). The means of seven determinations carried out on the SCMK preparations are $105 \cdot 1$ μmoles thiol$+\frac{1}{2}$ disulphide/g protein and $85 \cdot 3$ μmoles thioglycollate/g protein (S.E.$=4 \cdot 1$ μmoles/g protein). The difference between the two means is $19 \cdot 8$ μmoles/g protein (S.E.$=5 \cdot 7$ μmoles/g protein), and this is significant at the 5 per cent. probability level. The data indicate that the polarographically determined thiol+disulphide content is as much as can be accounted for in terms of thioglycollate, while in several SCMK preparations there is a considerable excess.

TABLE 6

EFFECT OF CYANIDE TREATMENT ON THE THIOL+DISULPHIDE CONTENT OF WOOL PROTEIN PREPARATIONS AS DETERMINED BY THE AMPEROMETRIC NEOHYDRIN TITRATION

Nature of Preparation	Preparation Number	μmoles SH$+\frac{1}{2}$SS/g Protein		
		Before Treatment	After Treatment	Loss on Treatment
Purified SCMK* ..	Z58	39	13	26
SCMK	18	75	23	52
SCMK 2†	Z82F	11	6	5

* The *S*-carboxymethyl kerateine was prepared as follows: after thioglycollate extraction the protein was precipitated at pH $5 \cdot 0$, redissolved in thioglycollate, coupled with iodoacetate, and dialysed against water. The protein was precipitated out at pH $4 \cdot 1$, then redissolved again in alkali, and reprecipitated at pH $4 \cdot 1$ by dialysis. Altogether the protein was precipitated three times at pH $4 \cdot 1$ before a final dialysis against water. The solution was provided by Mr. J. M. Gillespie.

† This is a pool of 12 preparations provided by Mr. E. F. Woods.

The thiol content of a number of preparations was also examined. In all cases the disulphide predominated and the thiol content was under 20 per cent. of the sum total.*

The effect of cyanide treatment on the thiol and disulphide content of various wool protein preparations was also examined by amperometric titration (Table 6), and the results noted earlier in Table 4 were confirmed.

(d) *Catalytic Cobalt Wave Analysis*

From the effects of chemical treatment (Tables 4 and 6) it was considered possible that SCMK preparations contained measurable quantities of mixed disulphide. The presence of thiol+disulphide other than thioglycollate (Table 5)

* The presence of thiol does not necessarily conflict with the negative nitroprusside test given by all preparations after iodoacetate coupling. From the information given by Feigl (1947), it can be calculated that under the conditions used here, 20 μmoles thiol/g protein represent the lower limit of the sensitivity of the test.

could be interpreted as being due to cystine (not reduced by the thioglycollate treatment) or to cysteine (derived from incomplete conversion to the *S*-carboxymethyl derivative during iodoacetate coupling). Acid hydrolysis of an SCMK preparation might therefore be expected to result in :

(i) *S*-carboxymethyl cysteine being released unchanged following peptide bond breakage,

(ii) disproportionation of the mixed disulphide (before or after peptide bond rupture) to yield cystine and dithiodiglycollic acid, and

(iii) the release of any unreduced cystine or unreacted cysteine from the hydrolysed protein.

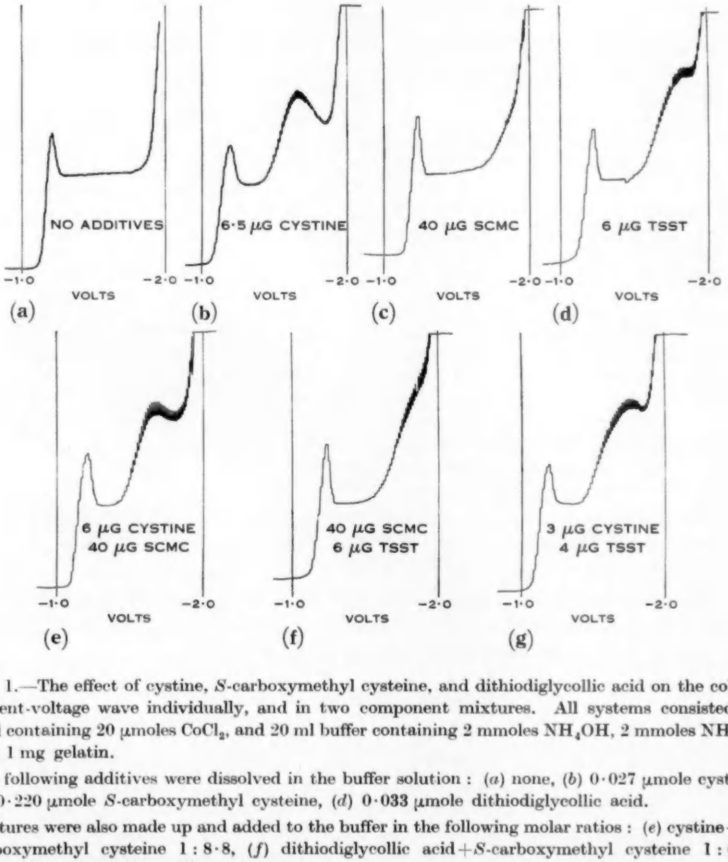

Fig. 1.—The effect of cystine, *S*-carboxymethyl cysteine, and dithiodiglycollic acid on the cobalt current-voltage wave individually, and in two component mixtures. All systems consisted of 1 ml containing 20 μmoles $CoCl_2$, and 20 ml buffer containing 2 mmoles NH_4OH, 2 mmoles NH_4Cl, and 1 mg gelatin.

The following additives were dissolved in the buffer solution : (*a*) none, (*b*) 0·027 μmole cystine, (*c*) 0·220 μmole *S*-carboxymethyl cysteine, (*d*) 0·033 μmole dithiodiglycollic acid.

Mixtures were also made up and added to the buffer in the following molar ratios : (*e*) cystine+*S*-carboxymethyl cysteine 1 : 8·8, (*f*) dithiodiglycollic acid+*S*-carboxymethyl cysteine 1 : 6·7, (*g*) cystine+dithiodiglycollic acid 1 : 1·8.

Full scale on the ordinates represents 15 μA.

For this reason the effects of various combinations of cystine, *S*-carboxymethyl cysteine, and dithiodiglycollic acid on the shape of the cobalt wave (Fig. 1 (*a*)) were investigated. It is evident that:

(i) each of the three compounds shows a characteristic wave pattern (Fig. 1 (*b–d*)),

(ii) *S*-carboxymethyl cysteine and dithiodiglycollic acid modify the cystine wave; the former by reduction and shifting of the $-1\cdot85$ V trough to $-1\cdot80$ V (Fig. 1 (*e*)), and the latter by shifting of the $-1\cdot70$ V peak to $-1\cdot75$ V and reduction of the $-1\cdot85$ V trough (Fig. 1 (*g*)),

(iii) *S*-carboxymethyl cysteine tends to eliminate the $-1\cdot85$ V point of inflection of dithiodiglycollic acid (Fig. 1 (*f*)).

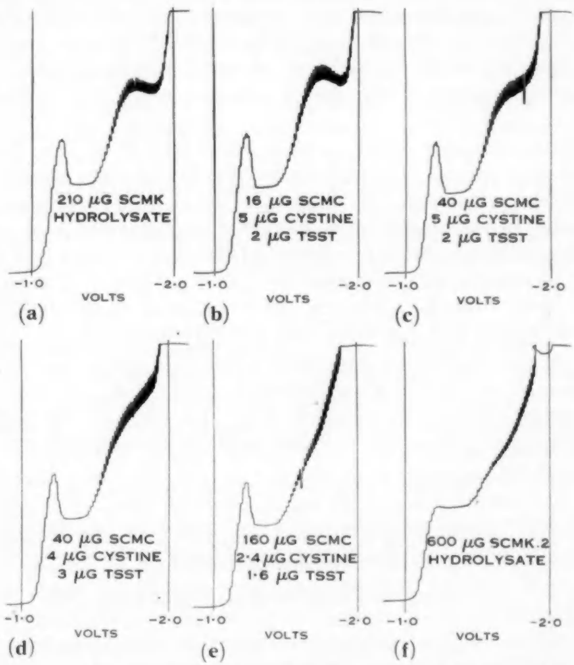

Fig. 2.—The effect of SCMK and SCMK 2 hydrolysates, and of three component mixtures of dithiodiglycollic acid, cystine, and *S*-carboxymethyl cysteine on the cobalt current-voltage wave. All systems consisted of 1 ml containing 20 μmoles $CoCl_2$, and 20 ml buffer containing 2 mmoles NH_4OH, 2 mmoles NH_4Cl, and 1 mg gelatin.

The following additives were dissolved in the buffer solution: (*a*) 210 μg SCMK hydrolysate (preparation 5) containing $0\cdot032$ μmole thiol+disulphide by polarography, and $0\cdot027$ μmole thioglycollate by isotopic assay; mixtures of dithiodiglycollic acid, cystine, and *S*-carboxymethyl cysteine in the following molar ratios: (*b*) 1 : 2 : 8, (*c*) 1 : 2 : 20, (*d*) 1 : 1 : 14, and (*e*) 1 : 1 : 100; (*f*) 600 μg SCMK 2 hydrolysate (preparation 21) containing $0\cdot007$ μmole thioglycollate by isotopic assay.

Full scale on the ordinates represents 15 μA.

X

It was found that the effect of an SCMK hydrolysate on the cobalt wave (Fig. 2 (*a*)) could be simulated by a system containing dithiodiglycollic acid, cystine, and *S*-carboxymethyl cysteine (Fig. 2 (*b*)). Taking the features of the two component systems (Fig. 1 (*e–g*)) into account, it was possible to approach the pattern obtained with an SCMK 2 hydrolysate (Fig. 2 (*f*)) by greatly increasing the proportion of *S*-carboxymethyl cysteine (Fig. 2 (*c–e*)), but retaining both dithiodiglycollic acid and cystine.*

IV. Discussion

The evidence presented in Tables 1 to 3 shows that all the soluble wool proteins examined contain sulphur derived from thioglycollate.† It would appear that physical adsorption is probably not a major factor, since even a large excess of cold thioglycollate does not produce a substantial reduction in the ^{35}S content (Table 4). Furthermore, it is unlikely that the thioglycollate is held to the protein by hydrogen bonds or metal chelation, since neither urea, nor ethylenediaminetetra-acetic acid treatments result in the loss of radioactivity.

The partial removal of radioactivity from SCMK by the trichloroacetic acid treatment (Table 2) may possibly be explained by the solubilization of a more highly labelled portion of SCMK such as the high sulphur containing protein (Gillespie 1958) in the organic phase following trichloroacetic acid precipitation. Other proteins, notably serum albumin (Levine 1954; Korner and Debro 1956) are soluble in organic solvents under these circumstances. This solubilization could have been reduced by washing the precipitates at low temperature, but partial solubilization may not have been entirely eliminated.

If the mixed disulphide β-carboxy-β-aminoethyl carboxymethyl disulphide occurs in extracted wool proteins (Tables 4 and 6), then by analogy with the earlier described systems (Kolthoff, Stricks, and Kapoor 1955; Eldjarn and Pihl 1957), it would arise as an intermediate in the overall reaction

$$\text{W-S-S-W} + 2\text{TSH} \rightleftharpoons 2\text{WSH} + \text{T-S-S-T},$$

where W-S-S-W represents unreduced wool, and TSH the thioglycollic acid. The first step in such a reaction can be represented by

$$\text{W-S-S-W} + \text{TSH} \rightleftharpoons \text{WSH} + \text{W-S-S-T}.$$

In view of the reversibility of the above reactions, it is likely that the smaller amount of label associated with SCMK 2 (Table 1) may be due to the use of multiple extractions and to the large overall liquor : wool ratio (180 : 1) used.

* One obvious difference between Figures 2 (*e*) and 2 (*f*) is in the absence of the —1·20 V peak in the latter. This particular maximum is characteristic of the cobalt wave, and has already been partially suppressed with gelatin (Fig. 1 (*a*)). It is possible to suppress this peak completely as in Figure 2 (*f*) by simply increasing the amount of gelatin in the system. Presumably at the high concentration of the SCMK 2 hydrolysate used, a similar suppression is observed. However, this region of the current-voltage curve is without significance in the present argument.

† It has generally been assumed that the sulphur derived from thioglycollate is in the form of thioglycollate. However, as will be discussed later, this may not apply to all the sulphur.

By contrast, more label may be attached to SCMK (Table 2) because only a single extraction is involved and the liquor : wool ratio used is only 30 : 1. If a figure of 500 μmoles cystine/g of wool (Human 1958) is assumed, then the molar ratios of thioglycollate : cystine are 36 : 1 and 6 : 1 respectively. The effect of increasing the thioglycollate : cystine ratio, even without further incubation at 50 °C, is shown (Table 3) to result in a reduced label content of the SCMK.

While it appears that SCMK 2 could contain up to 5 μmoles of mixed disulphide/g protein, it is clear that sulphur is also present in another form to the extent of about 3–6 μmoles/g protein (Tables 4 and 6). Similarly SCMK contains material which resists all attempts at removal, and which is present in somewhat larger quantities (13–23 μmoles/g protein). The gelatin experiments were undertaken in an effort to throw further light on this question. However, even in this case some 7–10 μmoles/g protein are irreversibly bound to the gelatin. This amount of thioglycollate could theoretically be bound as mixed disulphide only if the higher estimate for the cystine content of gelatin* is accepted and if most of the half-cystine present were converted to such a form. The latter assumption is unlikely in view of the fact that the greatest amount of thioglycollate associated with SCMK could have involved only 14 per cent. of the total available half-cystine residues. Furthermore, no evidence for disulphide (or thiol) could be found in the gelatin by amperometric titration (neohydrin and mercuric chloride methods) or any other means. It seems likely then that gelatin in common with extracted wool proteins retains thioglycollate derived sulphur in some unknown form.

It is quite possible that the content of this residual sulphur is higher than the above estimates suggest. One reason for believing this is that since there is fair agreement between amperometric and isotopic estimates in the case of SCMK 2 (Table 5), one might expect that neohydrin treatment in the presence of sulphite, followed by dialysis, would result in complete discharge of radioactivity. However, 6–7 μmoles/g protein, which were presumably not estimated polarographically, are still retained, as the ^{35}S content shows.

Several possibilities can be considered regarding the nature of this residual sulphur. In the first place some relatively small amount of impurity of a non-thioglycollate nature may either be present in the labelled thioglycollate as supplied, or it may have arisen during the warm alkaline treatment employed for the protein extraction.† If, however, thioglycollate were involved, then attachment through its carboxyl group alone by either salt-linkages or even peptide bonds is considered unlikely, since the former would not explain the irreversible nature of the attachment, while the formation of the latter would

* According to the highest estimate available (Neuman 1949) commercial gelatin may contain up to 7 μmoles cystine/g protein. However, other estimates (Eastoe 1955) put the figure at less than 2 μmoles/g gelatin.

† An indication that thioglycollate does undergo chemical modification under the conditions of wool protein extraction was obtained by Gillespie (unpublished data). He found that the thioglycollate solutions develop an absorption at 330 mμ ; after prolonged standing the solutions became visibly yellow.

not be favoured under alkaline conditions.* Attachment through the thiol group could account for the unreactive nature of the residual sulphur. A thiol ester linkage with the carboxyl group of glutamic and/or aspartic acid side chains is a possibility, although under the alkaline conditions of the protein extraction the equilibrium almost certainly favours hydrolysis rather than esterification (Connor 1953). Another possibility is the formation of sulphenic acid esters with the hydroxyl groups of serine, threonine, or tyrosine residues; however, these compounds are also probably unstable in alkaline media. It is concluded that none of the alternatives discussed above is entirely satisfactory, and it is suggested that clarification may result from further studies such as using chemically modified wools and ^{14}C-labelled thioglycollic acid.

The presence of excess thiol + disulphide over thioglycollate in some SCMK preparations (Table 5) finds confirmation in the studies of the catalytic cobalt wave (Figs. 2 (*a*) and 2 (*b*)). The latter shows that systems containing cystine and dithiodiglycollate† in a 2 : 1 molar ratio simulate the composition of the SCMK hydrolysate. The mixed disulphide would require only a 1 : 1 ratio, so that an excess of cyst(e)ine is indicated. By contrast SCMK 2 preparations and SCMK preparations in which a pH 5·0 precipitation and redissolution in thioglycollate was undertaken (preparation 22, Table 5) appear to contain little if any excess cyst(e)ine. In the case of SCMK 2 hydrolysates a 1 : 1 cystine : dithiodiglycollic acid ratio proved the closest match in the catalytic cobalt wave experiments (Figs. 2 (*e*) and 2 (*f*)). However, both the shape and position of the catalytic waves may be influenced by concentration, as well as by traces of interfering substances. For this reason it is preferable to regard the results obtained by this method as indicating the manner in which waves resembling those of SCMK and SCMK 2 may be produced and not as implying that the compositions of these two fractions are thereby established.

There is some uncertainty regarding the nature of the thiol + disulphide other than thioglycollate in SCMK. In two cases at least (preparations 27 and 31, Table 5), there is no evidence for the presence of thiol. On the other hand in preparations 30 and 32 there is almost enough thiol present to account for the thiol + disulphide content, while other preparations are intermediate. One complicating feature is that the thiol referred to in Table 5 may be due in part or wholly to thioglycollate, although it is difficult to see by what means this thioglycollate could be attached to the protein. Leaving aside preparation 14, which was not studied in detail and which may not be strictly comparable to other preparations, it appears that SCMK could contain up to 19 μmoles cystine/g protein, but that the presence of cysteine up to 19 μmoles/g protein is uncertain. On the other hand, some preparations could contain both cystine and cysteine in varying proportions.

* It is of interest that Bradley and Easty (1953) in their study of mandelic acid binding by wool suggest that a salt-link is responsible; however, examination of the results in Table 3 of their paper indicates that dilute aqueous ammonia treatment failed to remove the last 60–80 μmoles mandelic acid/g protein. The attachment of this residual amount by other means, for example, through the hydroxyl group, is therefore a possibility.

† It is not possible to distinguish between cystine and cysteine or thioglycollate and dithiodiglycollate in these experiments.

The presence of mixed disulphide is relevant to a number of problems associated with wool protein research. In the first place it is now evident that mixed disulphide formation can be brought about in wool under a number of conditions, and that it is not confined to soluble wool fractions. Schöberl (1953) provided evidence for mixed disulphide formation as a result of the oxidative step in the cold-wave process, while wool which has been permanently set with ammonium thioglycollate (Farnworth 1957), has also been shown to contain the mixed disulphide (Springell 1958). It is of interest in this connection that Schöberl and Gräfje (1958) have intentionally converted the cystine disulphide in wool to the mixed disulphide by reacting reduced wool with carboxymethyl carboxymethane thiolsulphinate.

It has been shown from time to time in these laboratories that various wool protein preparations which had been coupled with iodoacetic acid contain residual disulphide, as exemplified by recent work of Gillespie (1958). Such disulphide was detected by the Shinohara (1935) method and has been generally attributed to unreduced cystine. It now appears that at least some of this disulphide could be in the form of the mixed disulphide. Furthermore, one difference between the two low sulphur containing proteins, SCMK 2 and the SCMK protein isolated by Gillespie (1958), may lie in the amount of bound thioglycollate and thus of mixed disulphide. It is too early to speculate whether this factor alone could account for the differences in properties between the two preparations.

Although it was initially hoped to throw some light on the wool protein sulphur which cannot be accounted for by Gillespie (1958), Simmonds (1955) and others in this laboratory in terms of *S*-carboxymethyl cysteine + methionine + cystine + cysteine sulphur, it now seems likely that the thioglycollate sulphur contribution is included in Gillespie's " cystine " values.

Attempts to isolate a strictly homogeneous protein from wool in these laboratories may not have been successful so far, since the electrophoretic examination of purified wool protein preparations is complicated by anomalous patterns, and solubility tests (Gillespie 1957) are not clearly indicative of homogeneity. Aggregation as a possible factor contributing towards electrophoretic heterogeneity has been discussed by Gillespie (1956) and Woods (1959). Other possible reasons for the heterogeneity such as racemization during extraction (Lindley and Swan, unpublished data) have been considered. By analogy with recent work on ribonuclease (White 1958), it is suggested that the presence of mixed disulphides may be yet another factor which could contribute towards heterogeneity. A further consequence of such a relatively labile disulphide bond (Schöberl, Tausent, and Wagner 1954) in the protein molecule may be in providing a reactive centre. It is conceivable that, under relatively mild conditions, the following disproportionation reaction might take place :

$$2\text{W-S-S-T} \rightarrow \text{W-S-S-W} + \text{T-S-S-T},$$

and which could result in new cross-link formation and aggregation.

A point which is becoming increasingly evident in the course of studies of wool proteins with radioisotopes is that the last traces of additives used at various stages of preparation are very difficult to remove. Apart from the instance of

the residual sulphur referred to in this work, Gillespie and Springell (1957) found that zinc used in fractionation experiments was removed only after lengthy dialysis. The presence of even small amounts of such previously unsuspected impurities could adversely affect physicochemical studies of wool proteins.

V. Acknowledgments

We wish to thank several of our colleagues for helpful discussion, Dr. S. J. Leach for a number of amperometric titrations with mercuric chloride, and Mr. W. B. Hall, Division of Mathematical Statistics, C.S.I.R.O., for statistical analysis.

VI. References

Biilman, E. (1905).—*Liebigs Ann.* **339**: 351.
Bradley, W., and Easty, G. C. (1953).—*J. Chem. Soc.* **1953**: 1519.
Brdička, R. (1933).—*Coll. Trav. chim. Tchécosl.* **5**: 238.
Connor, R. (1953).—" Organic Chemistry." 2nd Ed. p. 835. (Ed. H. Gilman.) (John Wiley & Sons Inc.: New York.)
Dintzis, H. W. (1952).—Ph.D. Thesis, Harvard University.
Eastoe, J. E. (1955).—*Biochem. J.* **61**: 589.
Eldjarn, L., and Pihl, A. (1957).—*J. Biol. Chem.* **225**: 499.
Farnworth, A. J. (1957).—*Text. Res. J.* **27**: 632.
Feigl, F. (1947).—" Spot Tests." 3rd Ed. p. 226. (Elsevier Publishing Co.: Amsterdam.)
Gillespie, J. M. (1956).—*Proc. Int. Wool Text. Res. Conf. Aust. 1955* **B**: 35.
Gillespie, J. M. (1957).—*Aust. J. Biol. Sci.* **10**: 105.
Gillespie, J. M. (1958).—*Biochim. Biophys. Acta* **27**: 225.
Gillespie, J. M., and Lennox, F. G. (1953).—*Biochim. Biophys. Acta* **12**: 481.
Gillespie, J. M., and Lennox, F. G. (1955).—*Aust. J. Biol. Sci.* **8**: 97.
Gillespie, J. M., and Springell, P. H. (1957).—*Aust. J. Chem.* **10**: 344.
Goddard, D. R., and Michaelis, L. (1934).—*J. Biol. Chem.* **106**: 605.
Human, J. P. E. (1958).—*Text. Res. J.* **28**: 647.
Human, J. P. E., and Leach, S. J. (1956).—*Chem. & Ind.* **1956**: 149.
Kolthoff, I. M., Stricks, W., and Kapoor, R. C. (1955).—*J. Amer. Chem. Soc.* **77**: 4733.
Korner, A., and Debro, J. R. (1956).—*Nature* **178**: 1067.
Leach, S. J. (1959).—*Biochim. Biophys. Acta* **33**: 264.
Lennox, F. G. (1956).—*Proc. Int. Wool Text. Res. Conf. Aust. 1955* **B**: 22.
Levine, S. (1954).—*Arch. Biochem. Biophys.* **50**: 515.
Neuman, R. E. (1949).—*Arch. Biochem.* **24**: 289.
Schöberl, A. (1953).—*Naturwissenschaften* **40**: 390.
Schöberl, A., and Gräfje, H. (1958).—*Liebigs Ann.* **617**: 71.
Schöberl, A., Tausent, H., and Wagner, A. (1954).—*Naturwissenschaften* **41**: 426.
Shinohara, K. (1935).—*J. Biol. Chem.* **112**: 671.
Simmonds, D. H. (1955).—*Aust. J. Biol. Sci.* **8**: 114.
Springell, P. H. (1958).—*Text. Res. J.* **28**: 874.
Walkenstein, S. S., and Knebel, C. M. (1957).—*Analyt. Chem.* **29**: 1516.
White, F. H. (1958).—*Fed. Proc.* **17**: 334.
Woods, E. F. (1959).—*Aust. J. Biol. Sci.* **12**: 96.

SHORT COMMUNICATIONS

RECRYSTALLIZATION OF LEAD OXIDE FROM MOLTEN LEAD*

By D. H. Bradhurst† and A. S. Buchanan†

During an investigation of the surface tension of liquid lead by means of a sessile drop technique, small transparent yellow crystals were observed to form on the solidified surface after heating at 750 °C in an argon atmosphere. X-Ray crystallographic analysis, using a 9 cm Guinier camera, confirmed the tetragonal structure and lattice dimensions of yellow lead oxide. It seemed probable that the oxide had been formed from small amounts of oxygen in the cylinder argon used in these experiments. A more systematic study of this crystallization process was subsequently carried out *in vacuo*, using weighed additions of A.R. lead oxide. This note describes the recrystallization process observed.

Pure lead specimens (99·999%; supplied by the Metallurgy Department, University of Melbourne) consisting of 3·5 by 6·5 mm cylindrical pellets, were placed on specially prepared high density uranium dioxide plaques ($UO_{2\cdot00}$), and a weighed amount of A.R. lead oxide placed on the pellet. The assembly was inserted into an induction heated tube furnace which was then evacuated and heated to 750 °C. Photographs of the profile of the sessile drop were taken at different temperatures and time intervals. After each run, the plaque and lead surface were examined microscopically (×300) and photomicrographs were taken when necessary.

The process of solution of lead oxide was accompanied by random motion of the particles over the surface of the lead drop, in much the same manner as small particles of camphor move on a water surface. This effect was observed to occur for lead sulphide, lead selenide, and lead telluride also, although in these cases formation of surface crystals did not occur, a result probably associated with greater solubility in the lead. Plate 1 shows three photographs (Figs. 1–3) of the solution process with lead oxide, the motion of the particles producing a blurred image in Figure 2, for which a 60 sec exposure was used. The lowest temperature at which appreciable solution was observed lies in the range 500 to 530 °C for each of the four solutes.

The rate of cooling has some influence on the formation of the crystals. Plate 1, Figure 4, is a photomicrograph of a rapidly cooled sample, and it will be noted that the lead oxide crystal is irregular in shape and appears to have been formed by growth from a crack in the initially formed lead " crust ". Plate 1, Figure 5, on the other hand, shows some of the more regular lead oxide crystals produced by the slower cooling obtained in a furnace of larger heat capacity. These crystals were coloured red (probably an interference colour).

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† Chemistry Department, University of Melbourne.

The approximate maximum solubility of lead oxide in lead at 750 °C was estimated to be 0·019 molal (0·42 wt.%), which was inferred from the surface tension *v.* concentration isotherm (Bradhurst and Buchanan 1959, unpublished data), which showed maximum depression at this concentration. The value obtained by Richardson and Webb* was somewhat smaller (saturation at an interpolated value of 0·012 molal).

This work was supported by a grant from the Australian Atomic Energy Commission and forms part of a programme of work on the surface chemistry of liquid metal systems.

ACID–BASE EQUILIBRIA IN ACETIC ACID AT ELEVATED TEMPERATURES†

By R. J. L. Martin‡ and I. H. Reece‡

A number of acid-base equilibrium constants in acetic acid at 25 °C have been determined by Lemaire and Lucas (1951), Smith and Elliott (1953), and Kolthoff and Bruckenstein (1956) using spectrophotometric measurements. Kolthoff and Bruckenstein (1956) have also determined the indicator constant for *p*-naphtholbenzein with *p*-toluenesulphonic and hydrochloric acids in the temperature range 18–33 °C. From a consideration of these results and those of Jolly (1952) on the heat of neutralization of perchloric acid and sodium acetate, Kolthoff and Bruckenstein (1956) conclude that the heats of reaction between acids and bases in acetic acid are of the order of 5 to 7 kcal mole^{-1} and are very little affected by the strength of the acid or the base, that is,

$$B+H^+X^- \rightleftharpoons BH^+X^-, \quad 5 \text{ to } 7 \text{ kcal mole}^{-1}.$$

The equilibrium constant for the reaction between perchloric acid and the base, water, has been determined at 25 °C by Lemaire and Lucas (1951), Smith and Elliott (1953), and Kolthoff and Bruckenstein (1956) while that for acetamide has been determined by Lemaire and Lucas (1951). However, the values for these constants were required at 105 °C, which is well outside the temperature range used by Kolthoff and Bruckenstein (1956), and any extrapolation to 105 °C using their estimated heat of reaction would give only approximate values for the equilibrium constants.

Smith and Elliott (1953) and Kolthoff and Bruckenstein (1956) for most of their work used *p*-naphtholbenzein. At 25 °C the acid form of this indicator is green and has absorption maxima at 464 and 625 mμ. At 105 °C or on heating

* Richardson, F. D., and Webb, L. E. (1955).—*Trans. Inst. Min. Metall. Lond.* **64** : 529.

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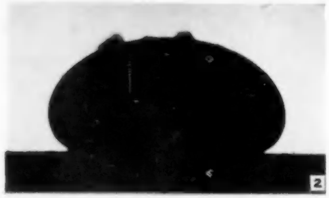
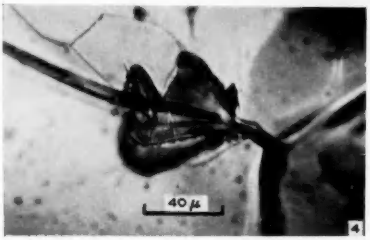
‡ School of Chemistry, University of New South Wales, Broadway, Sydney.

RECRYSTALLIZATION OF LEAD OXIDE FROM MOLTEN LEAD





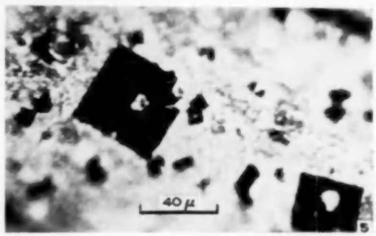


Fig. 1.—Photograph of sessile drop of liquid lead on uranium dioxide plaque at 380 °C, with sample of undissolved lead oxide at apex. ×10. Fig. 2.—The same sample at 510 °C. ×10. The blurred image was produced by motion of lead oxide particles on the lead surface. Fig. 3.—Clean profile of sessile drop at 740 °C after complete solution of lead oxide. ×10. Figs. 4 and 5.—Photomicrographs of lead oxide crystal; 4 produced by rapid cooling and 5 by slow cooling of the lead sample.

we found that the acid form undergoes an irreversible change from green to orange-brown with a green fluorescence. This is very well illustrated in Figure 1, where it will be seen that at 105 °C the acid form has absorption maxima at 470, 500, and 625 mμ. Furthermore, on continued heating at this temperature the intensity of the absorption at 625 mμ decreases, while that at 475 and 500 mμ simultaneously increases. Smith and Elliott (1953) have reported that this indicator obeys Beer's law only for concentrations less than 5×10^{-5}M. Possibly the divergence may be attributed to the slow acetylation of this tertiary alcohol

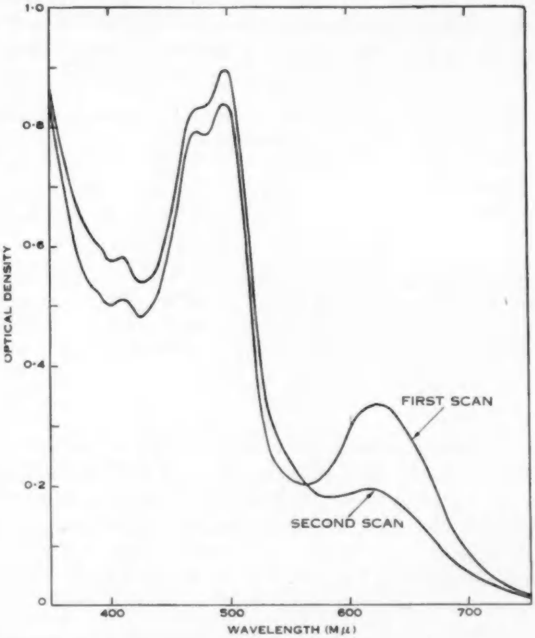


Fig. 1.—Two successive scans of the solution containing *p*-naphtholbenzein ($1 \cdot 13 \times 10^{-4}$M), perchloric acid (0·041M), and water (0·0041M) in acetic acid in a 1·00 cm cell at 105 °C.

at higher concentrations. At the higher temperatures the rate of acetylation increases very rapidly. For this reason determinations were made using 2,4-dinitro-*NN*-diethylaniline as indicator.

It was found that 2,4-dinitro-*NN*-diethylaniline, a tertiary amine, was a completely stable and satisfactory indicator for the conditions used in these experiments. Although Lemaire and Lucas (1951) determined its indicator constant, they did not use it for the determination of acid-base equilibrium constants. The acidic form is colourless, and the basic form has an absorption maximum at 370 mμ at 105 °C with a molar extinction coefficient of 13,340. The addition of the base, sodium acetate, produces no change in the molar

extinction coefficient indicating that the 2,4-dinitro-*NN*-diethylaniline in acetic acid exists completely in the basic form. This indicator obeyed Beer's law for concentrations at least up to $1 \cdot 7 \times 10^{-4}$M.

The equilibrium constants for the reactions between perchloric acid and the bases, indicator, water, and acetamide at 105 °C are given in Table 1 and are the mean values of at least a dozen determinations. For comparison, the values for these equilibrium constants as determined by Lemaire and Lucas (1951) at 25 °C are also given in Table 1. For an 80 °C rise in temperature the constants increase by a factor varying between four- and tenfold. The heats of reaction are of the same order of magnitude as predicted by Kolthoff and Bruckenstein (1956) and show a twofold variation in magnitude among themselves.

TABLE 1

ACID-BASE EQUILIBRIUM CONSTANTS IN ACETIC ACID AT VARIOUS TEMPERATURES WITH PERCHLORIC ACID

Base	K (mole l^{-1})		ΔH (kcal mole^{-1})
	25 °C	105 °C	
2,4-Dinitro-*NN*-diethylaniline	$6 \cdot 55 \times 10^{-5}$	$6 \cdot 50 \times 10^{-4}$	6·4
Water	$3 \cdot 03 \times 10^{-2}$	$11 \cdot 4 \times 10^{-2}$	3·7
Acetamide	$1 \cdot 49 \times 10^{-4}$	$8 \cdot 60 \times 10^{-4}$	4·9

Experimental

The acetic acid was purified and the stock solution of perchloric acid prepared by the methods previously used (Martin 1957). B.D.H. *p*-naphtholbenzein was used without any purification. 2,4-Dinitro-*NN*-diethylaniline was prepared from 2,4-dinitrochlorobenzene and diethylamine (Lemaire and Lucas 1951). It was crystallized from both ethanol and methanol and had m.p. 79·3–80·2 °C (uncorr.). Stock solutions of the indicator were prepared on the same day that the spectrophotometric measurements were made and these were diluted to approximately $1 \cdot 7 \times 10^{-4}$M.

B.D.H. acetamide was crystallized by adding ether to a cold methanol solution (Wagner 1930). It was finally crystallized from chloroform and stored in a desiccator over fused calcium chloride. In the preparation of the stock solution the acetamide was always transferred from one vessel to another inside a dry-box.

All spectra were recorded on a Cary spectrophotometer Model 11 and 1·00 cm cells were used throughout.

The apparatus which was used for maintaining the test solution at the required temperature within the photometer is illustrated in Figure 2. The sample cell fitted snugly into a copper block maintained at 105 °C by thermal conduction across a copper-glass seal on the end of a glass tube through which hot oil was circulated. This equipment fitted through the cell compartment lid, from which it was thermally insulated and made light-tight. The most

suitable flexible material for handling the hot oil was found to be plastic garden hose. The temperature of the test solution was measured by means of a copper-constantan thermocouple junction which was placed between the cell and the enveloping copper block. Usually 15–25 min were required for the test solution to be heated to a constant temperature, which was also indicated by no further changes in the optical density.

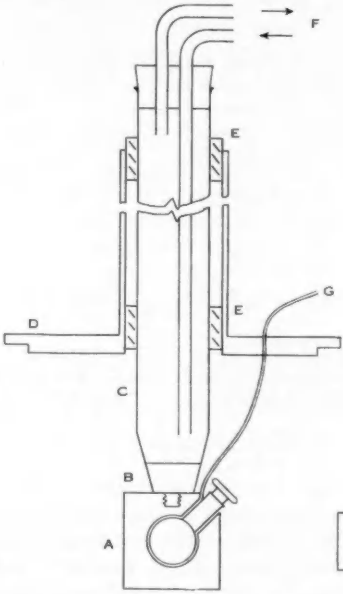


Fig. 2.—High temperature cell for a Cary spectrophotometer. *A*, Quartz cell (1 cm) mounted in a copper block ; *B*, glass to copper seal threaded into the copper block ; *C*, glass tube as hot oil reservoir ; *D*, brass lid of cell compartment ; *E*, insulating cork spacers ; *F*, oil leads to thermostat ; *G*, thermocouple leads passing through the lid.

For the calculation of the equilibrium constants the perchloric acid and its salts were assumed to be completely ionized into ion pairs. Any dissociation of the ion pairs into ions was shown by Bruckenstein and Kolthoff (1956) to be small and was therefore neglected.

$$HClO_4 \overset{\text{ionization}}{\rightleftharpoons} H^+ClO_4^- \overset{\text{dissociation}}{\rightleftharpoons} H^+ + ClO_4^-,$$

$$H_2O + H^+ClO_4^- \rightleftharpoons H_3O^+ClO_4^-,$$

$$CH_3CONH_2 + H^+ClO_4^- \rightleftharpoons [CH_3C(OH)N^+H_2]ClO_4^-,$$

$$I + H^+ClO_4^- \rightleftharpoons IH^+ClO_4^-,$$

or generally,

$$B + H^+ClO_4^- \rightleftharpoons BH^+ClO_4^-.$$

The equilibrium constant

$$K=[\mathrm{B}][\mathrm{H^+ClO_4^-}]/[\mathrm{BH^+ClO_4^-}].$$

The heat of reaction $-\Delta H$ was calculated using the integrated form of the van't Hoff isochore

$$\log K_1-\log K_2=-\frac{\Delta H}{2\cdot 303R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right).$$

References

Bruckenstein, S., and Kolthoff, I. M. (1956).—*J. Amer. Chem. Soc.* **78**: 2974.
Jolly, W. L. (1952).—*J. Amer. Chem. Soc.* **74**: 6199.
Kolthoff, I. M., and Bruckenstein, S. (1956).—*J. Amer. Chem. Soc.* **78**: 1.
Lemaire, H., and Lucas, H. J. (1951).—*J. Amer. Chem. Soc.* **73**: 5198.
Martin, R. J. L. (1957).—*Aust. J. Chem.* **10**: 268.
Smith, T. L., and Elliott, J. H. (1953).—*J. Amer. Chem. Soc.* **75**: 3566.
Wagner, E. C. (1930).—*J. Chem. Educ.* **7**: 1135.

THE KINETICS OF ACID AND ALKALINE HYDROLYSIS OF *p*-NITROPHENYL MONO-*O*-METHYL-β-GLUCOPYRANOSIDES*

By M. A. Jermyn†

In the course of other work I showed qualitatively (Jermyn 1957*b*) that *p*-nitrophenyl 2-*O*-methyl-β-glucopyranoside is much less susceptible to alkaline hydrolysis than the unmethylated parent compound or glucosides *O*-methylated elsewhere on the glucopyranoside ring. These results agreed with those of McCloskey and Coleman (1945) on phenyl 2,3-di-*O*-methyl-β-glucopyranoside and Bardolph and Coleman (1950) on phenyl 2-*O*-methyl-β-glucopyranoside. These workers showed these 2-*O*-methylated β-glucosides to be almost unaffected by alkaline conditions that completely converted the parent phenyl glucoside to laevoglucosan (1,6-anhydro-β-glucose).

Subsequent workers have in general agreed with the hypothesis of Coleman and his collaborators that these results are to be explained by the initial formation with Walden inversion of a 1,2-epoxide ring in the alkaline hydrolysis of β-glucosides, which ring is subsequently ruptured with a second inversion of configuration to give laevoglucosan. *O*-Substitution on carbon 2 in β-glucosides would prevent or at least hinder these reactions.

The mechanism of acid hydrolysis of glycosides is quite different from that of alkaline hydrolysis and influenced by the many factors discussed by Shafizadeh (1958). The combined influences of ring conformation, group interaction, and

* Manuscript received January 22, 1959.

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shielding effects make useful predictions of the effect of substitution on hydrolysis rate very difficult, and in fact very little work has been done in the field. Shafizadeh illustrates one of the few valid generalizations—that 2-deoxyglycosides are much more rapidly hydrolysed than the parent compound—but there is no information about how 2-oxy-substituted glycosides should behave.

Since the hydrolysis of nitrophenyl glycosides lends itself to rapid and accurate measurement, it seemed of interest to give a quantitative description of the observed differences in reaction rates. The kinetics of the acid and alkaline hydrolysis of nitrophenyl glycosides have been studied by Snyder and Link (1952), who included *p*-nitrophenyl β-glucopyranoside amongst the substrates that they used.

The glycosides were prepared in this laboratory (Jermyn 1954, 1957*a*). Acid or alkaline solutions and aqueous solutions of the glucosides were preheated to the desired temperature and mixed to give the required final concentrations of the reactants. Alkaline hydrolysis was followed colorimetrically from the amount of *p*-nitrophenol produced (Snyder and Link 1952) by direct reading on the reaction mixture; acid hydrolysis, by withdrawing samples and mixing with enough concentrated Na_2HPO_4–Na_3PO_4 solution to bring the mixture finally to pH 9.

The fraction of the glucosides decomposed during the experimental period was never more than 2 per cent. and was usually much less; under these conditions the approximation involved in considering the rate of *p*-nitrophenol liberation as linear with time would lead to errors much smaller than experimental error. These linear rates have been used as the equivalent of initial reaction rates.

Second order velocity constants, k, were calculated from the equation k=initial rate/[glucoside][H^+ or OH^-] and the energies of activation from the velocity constants at three temperatures over the ranges given. Reaction rates at $40 \pm 0 \cdot 1$ °C were used in comparative studies and entropies of activation have been calculated for this temperature.

Alkaline Hydrolysis

The alkaline hydrolysis of the 4- and 6-*O*-methyl and the unmethylated *p*-nitrophenyl β-glucopyranoside followed the expected second order kinetics, the initial reaction rate being proportional both to potassium hydroxide ($0 \cdot 05$ to $0 \cdot 5$N) and glycoside (10^{-4} to 10^{-3}M) concentrations. The temperature dependence of the second-order velocity constants was normal, the plot of $1/T$ (abs.) against $\log k$ being linear from 20 to 40 °C.

Although the rate of alkaline hydrolysis of the 2-*O*-methylglucoside was also directly proportional to glycoside concentration, it became apparent that the same relationship did not hold for potassium hydroxide in the high concentrations (2 to 10N) needed to get measurable rates. From Figure 1 it can be seen that these rates are in fact proportional to $[KOH]^3$, and velocity constants were calculated from the fourth-order relation,

$$\text{Observed rate} = k\,[\text{glycoside}][\text{KOH}]^3.$$

This equation has been put in terms of [KOH] to mark the fact that, at the concentrations used, the approximation $a_{OH^-} = [OH^-]$, which justifies using $[OH^-]$ in not very refined kinetic calculations, has no longer any validity. The complex relationship between a_{OH^-} and [KOH] may in fact partially determine the complexity of the kinetics. The temperature dependence of these constants was normal in the range 30 to 50 °C.

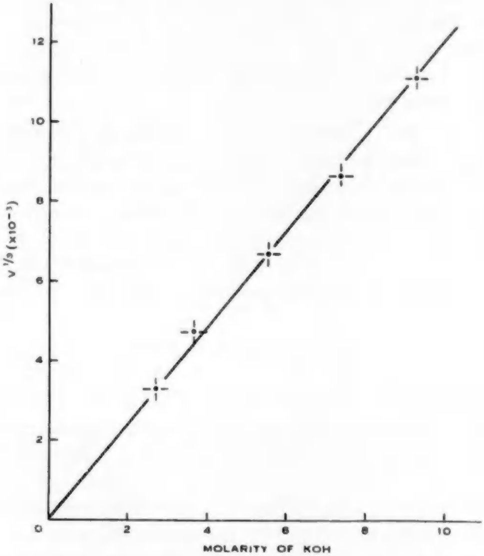

Fig. 1.—Dependence of the rate of hydrolysis of 10^{-3}M *p*-nitrophenyl 2-*O*-methyl-β-glucopyranoside at 40 °C on potassium hydroxide concentration. The initial reaction velocities (*V*) were originally expressed in terms of *p*-nitrophenol liberated as mol l^{-1} min^{-1}.

Acid Hydrolysis

The kinetics of hydrolysis were second order in all four cases, being proportional to glycoside (5×10^{-4} to 3×10^{-3}M) and hydrochloric acid (0·1 to 0·5N) concentrations. The temperature dependence of the second-order constants so obtained was normal in the range 30 to 50 °C.

The complete data for acid and alkaline hydrolysis are summarized in Table 1.

Snyder and Link (1952) showed that the acid and alkaline hydrolysis of any given nitroaryl glycoside were both kinetically second-order reactions, whatever the mechanisms involved. Comparing the numerical values of the kinetic constants for the two types of hydrolysis of different glycosides, they found that the observed scatter in the magnitude of the reaction rates was due to differences in " shape factors " (i.e. the sort of differences here expressed more

precisely in terms of entropy of activation) rather than in energies of activation. These conclusions are confirmed here; the slight differences in the values for the energy of activation for the only compound studied in both papers probably reflect differences in experimental techniques and conditions.

The absence of *p*-nitrophenyl 3-*O*-methyl-β-glucopyranoside from the list of substances studied in this work was due to shortage of material. I have shown (Jermyn 1957*a*, 1957*b*) that 3-*O*-methyl substitution, like 4- and 6-*O*-methyl substitution but unlike 2-*O*-methyl substitution, makes no qualitative

TABLE 1

KINETIC DATA FOR THE ACID AND ALKALINE HYDROLYSES OF UNMETHYLATED AND *O*-METHYLATED *p*-NITROPHENYL β-GLUCOPYRANOSIDES

Substance	Acid Hydrolysis			Alkaline Hydrolysis		
	Energy of Activation (30–50 °C) (cal mol^{-1})	k (40 °C) (l mol^{-1} min^{-1})	Entropy of Activation (ΔS_c^*; 40 °C) (cal deg^{-1})	Energy of Activation (20–40 °C) (cal mol^{-1})	k (40° C) (l mol^{-1} min^{-1})	Entropy of Activation (ΔS_c^*; 40 °C) (cal deg^{-1})
p-Nitrophenyl β-glucopyranoside	28,000*	$3 \cdot 93 \times 10^{-5}$†	14·1	24,300‡	$5 \cdot 23 \times 10^{-3}$§	12·0
2-*O*-methyl-β-glucopyranoside	26,800	$4 \cdot 55 \times 10^{-5}$	8·5	21,600‖	$1 \cdot 52 \times 10^{-6}$¶	14·9
4-*O*-methyl-β-glucopyranoside	29,300	$3 \cdot 70 \times 10^{-5}$	18·4	25,900	$4 \cdot 02 \times 10^{-3}$	26·0
6-*O*-methyl-β-glucopyranoside	33,300	$4 \cdot 09 \times 10^{-5}$	31·1	22,600	$3 \cdot 42 \times 10^{-3}$	5·8

* Snyder and Link (1952) give 25,500 cal mol^{-1} based on values of k at 65 and 85 °C only.

† From Snyder and Link's (1952) data, k (second order calc.) $= 4 \cdot 2 \pm 0 \cdot 11 \times 10^{-4}$ l mol^{-1} min^{-1} at 65 °C, $3 \cdot 51 \pm 0 \cdot 062 \times 10^{-3}$ at 85 °C.

‡ 25,800 (Snyder and Link (1952), 45–65 °C).

§ $7 \cdot 40 \pm 0 \cdot 35 \times 10^{-3}$ at 45 °C (Snyder and Link 1952).

‖ 30–50 °C.

¶ Fourth-order constant, units are l^3 mol^{-3} min^{-1}.

difference to the alkaline hydrolysis of *p*-nitrophenyl β-glucopyranoside. Bardolph and Coleman (1950) have likewise shown the slight effect of 3-*O*-methyl substitution on phenyl β-glucopyranoside. It therefore seems unlikely that anything more than the quantitative differences in an unchanged reaction mechanism found for the 4- and 6-*O*-methylglucosides would have been discovered.

The exception to uniformity is the alkaline hydrolysis of the 2-*O*-methylglucoside. Substitution on carbon 2 profoundly inhibits alkaline hydrolysis but not acid hydrolysis. The apparent fourth-order kinetics of the aberrant alkaline hydrolysis may possibly indicate a devious reaction mechanism, but in view of the profound influence of changes in ionic strength, dielectric constant

and a_{OH^-} due to the very high KOH concentrations employed, the kinetics would be expected to be complex in any case and no valid conclusions can be drawn on the data presented here. Substitution on carbon 6, which would preclude laevoglucosan formation, does not alter the kinetic picture, so that the primary process must involve carbon 2 in accordance with the ideas of McCloskey and Coleman (1945). The fact that the energy and entropy of activation are in the same range for both the normal and aberrant alkaline hydrolysis possibly means that the overall reactions are similar, whatever the paths traversed.

References

Bardolph, M. P., and Coleman, G. H. (1950).—*J. Org. Chem.* **15** : 169.
Jermyn, M. A. (1954).—*Aust. J. Chem.* **7** : 202.
Jermyn, M. A. (1957*a*).—*Aust. J. Chem.* **10** : 448.
Jermyn, M. A. (1957*b*).—*Aust. J. Chem.* **10** : 455.
McCloskey, C. M., and Coleman, G. H. (1945).—*J. Org. Chem.* **10** : 184.
Shafizadeh, Z. (1958).—*Advanc. Carbohyd. Chem.* **13** : 9.
Snyder, J. A., and Link, K. P. (1952).—*J. Amer. Chem. Soc.* **74** : 1883.

Corrigenda

Volume 12, Number 2

Page 219, structure III of the diagram : The heterocyclic ring should be five-membered as in structure II.

Page 288, structure II : *For* HO—C— *read* HO—C—H.